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Azathioprine use in
Inflammatory bowel disease

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Degree of Doctor of Medicine (MD)

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2003



Declaration

I hereby declare that the work in this thesis is original and undertaken by myself while working as a research fellow at The Gastrointestinal Unit, Western General Hospital, Edinburgh 1998-2001. I confirm that the thesis has not been submitted in candidature for any other degree, diploma or professional qualification

Dr Simon Campbell MB ChB MRCP (UK)

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Abbreviations

ARDS – Adult respiratory distress syndrome

ASD – Atrial septal defect

AZA – Azathioprine

CCF-IOIBD – Crohn's and Colitis Foundation of America / International organization
of inflammatory bowel disease

CD – Crohn's disease

CRP – C-reactive protein

CyA – Cyclosporin

DMSO – Dimethylsulphoxide

DNA – Deoxyribonucleic acid

EDTA - Ethylenediaminetetraacetic acid

F- Female

GETAID – Groupe d'Etude Therapeutique des Affections Inflammatoires Digestives

GCSF – Granulocyte colony stimulating factor

HBI – Harvey Bradshaw Index

HPLC – High performance liquid chromatography

HPRT – Hypoxanthine guanine phosphoribosyl transferase

IBD – Inflammatory bowel disease

IV – Intravenous

M- Male

MCV – Mean corpuscular volume

MWT – Mann-Whitney Test

NHL – Non-Hodgkin's Lymphoma

NVD – Normal vaginal delivery

PCP – Pneumocystis Carnii pneumonia

PBS – Phosphate buffer solution

PRPP - 5-phosphoribosyl 1-pyrophosphate

RBC – Red blood cell

SAH - S-adenosyl homocysteine

SAM - S-adenosyl methionine

tIMP- Inosine monophosphate

TPMT – Thiopurine-methyl-transferase

UC – Ulcerative colitis

WCC – White cell count

XO – Xanthine oxidase

5-ASA – 5-aminosalicylate

6-MP – 6-mercaptopurine

6-MMP – 6-methylmercaptopurine

6-MMR's – 6-methylmercaptopurine ribonucleotides

6-TG – 6-thioguanine

Publications arising from this thesis

Papers

Campbell S, Kingstone K, Ghosh S

Relevance of thiopurine methyltransferase activity in inflammatory bowel disease patients maintained on low-dose azathioprine

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Campbell S., Ghosh S.

Effective maintenance of IBD remission by azathioprine does not require concurrent 5-ASA

European Journal Gastroenterology & Hepatology 2001 Nov ;13: 1297-301

Campbell S., Ghosh S.

Is neutropenia required for effective maintenance of remission during azathioprine therapy in inflammatory bowel disease

European Journal of Gastroenterology & Hepatology 2001 Sep;13: 1073-6

Campbell S., Ghosh S.

Azathioprine use in Inflammatory bowel disease – Are we using it appropriately?

Inflammatory bowel disease monitor 2001 3(1); 2-9

Abstracts

Campbell S, Ghosh S

Combination immunomodulatory therapy with cyclosporin, azathioprine and corticosteroids in severe ulcerative colitis: The Edinburgh experience of outcome

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Relevance of thiopurine methyltransferase activity in inflammatory bowel disease patients maintained on low dose azathioprine

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Campbell S., Ghosh S

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rates in IBD patients ?**

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**Relevance of thiopurine methyltransferase activity in inflammatory bowel
disease patients maintained on low dose azathioprine**

British Society of Gastroenterology, Birmingham 2002

Abstract

Background:

Azathioprine (AZA) is an effective therapy for steroid dependent/resistant inflammatory bowel disease (IBD). Its optimal use is hampered by several areas of uncertainty. It is unclear whether neutropenia as an end-point of AZA therapy may be desirable. There are no data that confirm whether concurrent 5-acetyl salicylic acid (5-ASA) with AZA is beneficial in maintaining remission in IBD patients. *In-vitro* studies suggest that 5-ASA inhibits thiopurine methyltransferase (TPMT) and therefore increases AZA toxicity. Anecdotal reports of AZA use by women during pregnancy report low birth weight and skeletal abnormalities in their offspring. In male patients on AZA, chromosomal abnormalities and lower fertility have been reported. Low TPMT activity levels predispose to bone marrow suppression in patients treated with AZA. Furthermore, the level of TPMT activity has been shown to correlate with disease relapse rates in other medical conditions treated with AZA – but this has not as yet been reported in IBD patients. The role of AZA in maintaining remission in UC patients who have responded to intravenous cyclosporin (CyA) has not been specifically addressed.

Methods:

A retrospective database was created using data from 220 IBD patients who were taking or had taken AZA. Other data that were collected included dosage, concurrent medications, disease type and distribution, relapse rates, pregnancy and offspring details, surgery. Red cell TPMT activity was ascertained by radiochemical assay. Relapse rates per year of follow-up and time to first relapse were used to assess 4 main hypotheses :-

- 1) There is no difference in remission maintenance in IBD patients treated with AZA who achieve a neutropenic nadir neutrophil count compared with those who do not.
- 2) There is no difference in remission maintenance in IBD patients on concurrent 5-ASA + AZA therapy versus IBD patients on AZA therapy alone.
- 3) The total white cell count (WCC) and neutrophil count at 4 months do not correlate with IBD patients' respective nadir WCC and neutrophil count during AZA therapy.
- 4) There is no difference in remission maintenance in IBD patients treated with AZA when comparing those with low TPMT activity (<20 nmol/hour/ml) versus those with normal TPMT activity (>20 nmol/hour/ml).

The outcomes of pregnancies in IBD patients taking AZA and the outcomes of pregnancies of partners of male IBD patients taking AZA were reported upon. Finally the experience of long-term outcome of acute severe UC patients initially treated with IV CyA and then maintained on oral AZA was described.

Results:

Mean relapse rates per year of follow up for the non-neutropenic ($>2.5 \times 10^9$) group compared with the neutropenic group ($\leq 2.5 \times 10^9$) were not significantly different. Analysis was performed on UC and CD sub-groups and relapse rates were not significantly different. For 5-ASA, analyses were performed in UC, CD and IBD patient groups. There were no statistically significant differences between AZA alone and AZA+5-ASA groups. Patients who became neutropenic had a significantly lower mean TPMT activity than the mean TPMT activity of patients who developed other side effects. Patients on *low-dose* AZA had a significantly lower number of relapses patients if they also had a low TPMT level. In patients who responded to IV CyA, AZA therapy was beneficial in maintaining remission. Analysis of 25 pregnancies where one or other parent was on AZA at the time of conception did not show any unusual occurrence of birth defects in the newborn.

Conclusions:

Patients who developed a neutropenic nadir neutrophil count did not achieve increased disease remission compared with those who did not. Patients on concurrent 5-ASA with AZA do not appear to be afforded any benefit in terms of remission maintenance, but there were no excesses of clinically significant side effects with concurrent 5-ASA and AZA therapy. IBD patients treated with low dose AZA relapse less frequently if they have low TPMT activity levels. Offspring from IBD patients treated with AZA did not appear to have any obvious excess of birth defects, but the numbers of patients analysed were too small to make firm conclusions with regard to

the absolute safety of AZA during pregnancy. Although the numbers of CyA treated with concurrent AZA were small, the long-term outcomes of these patients support a view that concomitant use of CyA and is (relatively) safe and efficacious.

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Chapter 1 Azathioprine use in inflammatory bowel disease

1.1 Introduction

Azathioprine (AZA) therapy is an invaluable immunosuppressant used in the treatment for inflammatory bowel disease (IBD) as a second line drug. Optimal use of azathioprine and 6-mercaptopurine (6-MP) continues to be refined with our growing experience with its use. However, there are several areas of uncertainty in the use of AZA in IBD and this thesis aims to address some of these issues mainly from retrospective analysis of a large cohort of well characterised patients in Edinburgh.

AZA and 6-MP are purine analogues that interfere with nucleic acid metabolism and cell proliferation and thus have immunosuppressive properties. 6-MP was first synthesized in 1951 and initially used to treat leukaemia. Its pro-drug form AZA was produced in 1957, and used predominantly for preventing rejection in renal allografts(Murray et al. 1963). The first documented use of antimetabolite analogues in the treatment of IBD was in 1962 when a patient with chronic ulcerative colitis (UC) was successfully brought into remission with 6-MP(Bean 1962). Later, in 1969, AZA was used in a case where active Crohn's disease (CD) was successfully brought into remission(Brooke et al. 1969). Since then AZA and to a lesser extent 6-MP (the latter is used more widely in the US) are now regarded as the treatment of choice for IBD patients who are steroid refractory or dependent.

Since its first use in IBD, there have been numerous studies into the efficacy of the drug in both controlled and uncontrolled trials looking at remission induction and maintenance. Despite several conflicting reports, large meta-analysis have now confirmed both the safety and efficacy of the drug in maintaining remission, and answered several questions concerning dosage, and time to onset of action. Despite this the use of AZA remains less frequent than ideal - a recent Swedish study revealed that AZA accounts for as little as 2.2% of the annual drug prescriptions for CD patients and 0.5% for UC patients(Blomqvist et al 2001). In addition to this there is considerable heterogenicity between countries in their prescribing patterns of immunosuppressants(Meuwissen et al. 2000). For example, AZA is prescribed more often in Europe than North America, but the reverse is true for 6-MP.

At present, several questions remain unanswered with regard to the optimal use of AZA and 6-MP.

1.2 The purpose of the thesis

The purpose of this thesis was to address some of, as yet unanswered questions, concerning the use of AZA/6-MP therapy in the treatment of IBD. Specifically, five areas and key questions were chosen to be addressed by analysis of our patient database. The retrospective nature of the analysis would provide data that may be subject to biases and the conclusions therefore may require to be substantiated by prospective controlled studies, though the latter may not be possible for some of the questions.

1.2.1 Neutropenia: Is induction of modest neutropenia necessary for optimal maintenance of remission with AZA ?

It has long been recognized that immunosuppression of organ transplant patients with purine analogues sometimes caused potentially serious bone marrow suppression (Block, 1968; McGrath et al. 1975). This has meant that diligent blood count monitoring was required, and subsequently drug withdrawal was sometimes necessary when there was evidence of bone marrow suppression, in particular neutropenia. More recently this side effect of AZA/6-MP has now been looked at in a different light. In the treatment of IBD, there has been growing interest in the potential role of 'therapeutic neutropenia' as being a possible desirable end-point to such therapy. At present, AZA/6-MP dose is not routinely adjusted according to neutrophil count, unless dangerous levels of neutropenia occur. However, there have now been anecdotal reports of prolonged remission in IBD patients after life-threatening neutropenia caused by AZA/6-MP (Burke et al. 1989). There have also been reports of lower white cell counts and lower neutrophil counts in IBD patients who achieve remission earlier and achieve remission maintenance for longer (Colonna and Korelitz 1994; Korelitz et al. 1997). To investigate this question a retrospective study was performed on our IBD patients treated with AZA/6-MP and analysed by remission rates per year of follow-up, time to first relapse, and nadir neutrophil counts to elucidate whether neutropenia was of potential value in the treatment of IBD patients.

1.2.2 Concurrent 5-Aminosalicylate therapy(5-ASA): Is the use of concurrent 5-ASA therapy necessary for optimum maintenance of remission with AZA ?

The use of 5-aminosalicylates (5-ASA) in IBD patients predates the use of more powerful immunosuppressants such as AZA/6-MP. The efficacy of 5-ASA monotherapy in remission maintenance is accepted in the treatment of UC(Green et al. 1998), but probably has a more limited role in the maintenance treatment of CD(Sutherland et al. 1997). There is also some evidence that some 5-ASAs may have a therapeutic role in inducing remission in IBD patients(Biddle and Miner, Jr., 1990; Sutherland et al. 2000). Furthermore, there may be more long-term benefits of 5-ASA therapy, such as a reducing the overall risk of developing colonic carcinoma(MacGregor et al. 2000; Moghadasian et al. 1996).

In current clinical practise, concurrent use of purine analogues and 5-ASAs remains controversial and has not been systematically investigated. In-vitro work has suggested that benzoic acid derivatives such as 4 and 5-aminosalicylates inhibit the enzyme TPMT(Lowry et al. 1999; Szumlanski and Weinshilboum, 1995). This has led to concerns that patients taking concurrent 5-ASA medications with AZA/6-MP may be at increased risk of AZA/6-MP toxicity. Furthermore, it is unknown whether 5-ASA therapy in conjunction with AZA/6-MP has any beneficial effects on remission maintenance in IBD patients.

To answer some of these questions, this study looked at our IBD patients taking AZA/6-MP and analysed relapse rates and time to first relapse in these patients, stratifying them according to their 5-ASA status. In addition, documented side effects

while on AZA/6-MP therapy were analysed to evaluate the influence of concurrent 5-ASA therapy on these effects. However, the study did not specifically address the issue of development of colon cancer while on maintenance therapy, as there was no such patient on our database.

1.2.3 Pregnancy issues with azathioprine: Is the use of AZA at the time of conception safe regarding pregnancy outcome ?

Animal studies with AZA/6-MP have raised considerable concerns over the teratogenicity and clastogenicity of purine analogues. There have been numerous anecdotal reports of infections in neonates, skeletal and karyotypic abnormalities, increased spontaneous abortions and reduced birth weights from mothers who have been taking AZA/6-MP (Alstead et al. 1990; Evans et al. 1975; Williamson and Karp, 1981). Most of this experience has been from organ transplant patients who are often taking other concurrent immunosuppressives. Unfortunately, there has only been one report that has presented small series of outcomes of IBD patients taking AZA/6-MP (Alstead et al. 1990), and as such provided little information as to the clinical safety of AZA/6-MP with regards to potential offspring of patients taking AZA/6-MP. Finally, the effects of purine analogue therapy on the progeny of fathers who are taking such medication have received even less interest. A concern over fertility and possible clastogenicity in male patients remains (Eslami et al. 1976; Papoff et al. 1977; Salant et al. 1976). To address this, female IBD patients taking AZA/6-MP who had become pregnant were investigated and their experiences were reported. In addition, a postal survey was performed on male IBD patients taking AZA/6-MP to

give further information upon their progeny.

1.2.4 Thiopurine Methyltransferase activity (TPMT): How does TPMT activity levels in patients taking AZA relate to efficacy of maintenance therapy with AZA?

TPMT has received a lot of interest in recent years. Not only has it been recognized that low TPMT activity in patients leads to increase risk of developing neutropenia while taking AZA/6-MP, but there has been considerable interest in the genotypic and phenotypic variation of this enzyme and its activity (Corominas et al. 2000; Ishioka et al. 1999; Sebbag et al. 2000; Stolk et al. 1998). While it is recognized that patients with low TPMT activity should be given lower doses of AZA/6-MP or even avoid such treatment altogether, there has been concerns that patients with high TPMT activity may be more resistant to the immunosuppressants effects of AZA/6-MP therapy. Of particular interest is the fact that the majority of IBD patients in the UK get relatively modest doses of AZA/6-MP (often less than 2mg/kg). There have been many anecdotal reports in several centres of IBD patients being maintained in remission on even lower doses of AZA/6-MP. In this study the hypothesis that this apparent efficacy of low dose AZA/6-MP could be due to lower TPMT activity in these individual patients was tested. Initially a radiochemical assay to reliably measure TPMT activity was set up. After analysis results were pooled together, relapse rates were examined in these patients and stratified as to the dosage ($\leq 2\text{mg/kg}$ or $>2\text{mg/kg}$) and as to their measured TPMT activity ($\leq 20\text{u/hr/ml}$ or $>20\text{u/hr/ml}$).

1.2.5 *Cyclosporin (CyA) therapy in acute severe ulcerative colitis – the need for AZA: What is our single centre experience with the use of CyA and AZA as dual immunosuppressive therapy regarding efficacy and side effects ?*

Along the theme of immunosuppressants in the treatment of IBD, CyA therapy has re-emerged as an important agent for rescue therapy in acute severe UC. Initial reports were disappointing with 1 year relapse rates being particularly high (Kornbluth et al. 1994), advocating only a limited role of this intravenous therapy in light of the narrow therapeutic index CyA and its side-effect profile. However, more recently, there has been encouragement from the concomitant use of AZA in these patients. This has provided more encouraging remission maintenance (D'Haens et al. 2001). In this thesis the Edinburgh experience of outcome with this small group of UC patients was analysed and reported. This included dose regimens, AZA usage, surgery requirements, and side effects to add further important information for the role of CyA and AZA therapy. The limitations of such an observational study in a small series of patients is recognized but the reported experience with the use of CyA and AZA in the setting of UC remains quite limited.

Chapter 2 Literature Review

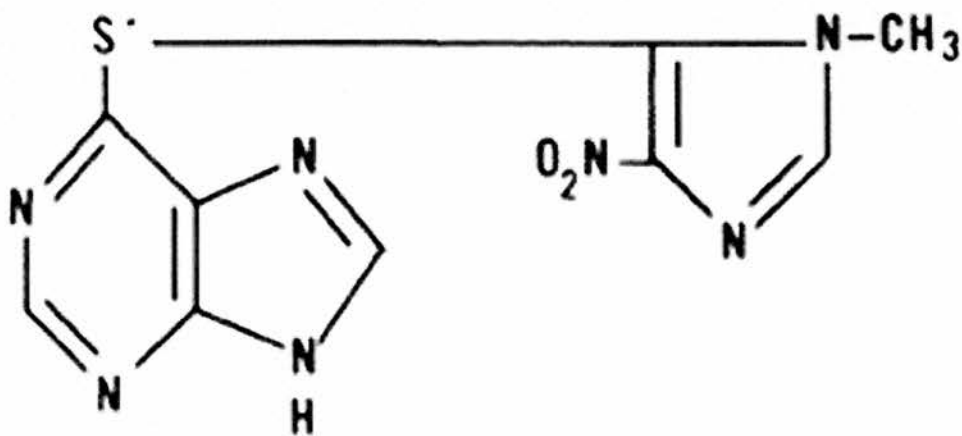
2.1 Pharmacology

AZA (6-(1-Methyl-4-nitroimidazol-5-ylthio)) is an imidazole derivative of 6-MP. It has the molecular formula $C_9H_7N_7O_2S$ and has a relative molecular mass of 277. The chemical structure of AZA and 6-MP are shown in Figure 2.1.

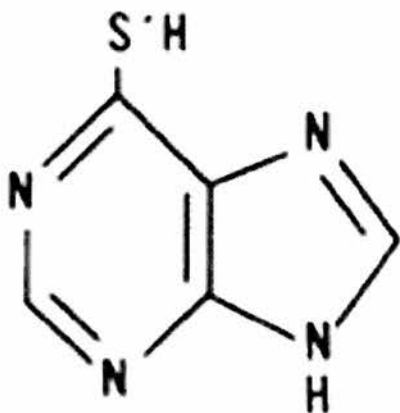
2.1.1 Pharmacodynamic properties – *In-vivo* AZA is rapidly converted to 6-MP and a methylnitroimidazole moiety by a non-enzymatic pathway dependent on glutathione and other sulphhydryl containing proteins – see Figure 2.2. Subsequently 6-MP is left available to the circulation. AZA is about 55% 6-MP by weight, and since over three-quarters of AZA is converted to 6-MP, 1mg of AZA is equivalent to 0.5mg 6-MP(Lennard, 1992).

Bioavailability of these drugs are low (less than 50%) due to the extensive catabolism by xanthine oxidase found in enterocytes and hepatocytes (but not haemopoietic tissue) (Lennard, 1998). 6-MP rapidly crosses the cell membranes and is converted intracellularly into a number of purine thioanalogues. There are 3 pathways; converted by xanthine oxidase into inactive thiouric acid (and renally excreted), converted by TPMT to inactive 6-methyl mercaptopurine (6MMP) or anabolized by hypoxanthine phosphoribosyl transferase (HPRT) to its active metabolites 6-thioguanine nucleotides (6TG) and 6-methylmercaptopurine ribonucleotides (6-MMPRs) (Elion 1969; Elion 1989). Up to 40% of 6TG is subsequently renally excreted (Elion 1989). This is summarized in figure 2.3. These enzymatic pathways are distinct and competitive and thus a lack of enzymatic activity at one point will result in an excess product being produced from a competing pathway.

Figure 2.1 – Chemical structure of Azathioprine and 6-mercaptopurine



AZATHIOPRINE



6-MERCAPTOPURINE

Table 2.1. Range of toxicity caused by Azathioprine (Present et al 1989)

<u>Toxic reaction</u>	<u>% Occurrence</u>
Infection	40%*
Bone Marrow Suppression	15%*
Hepatitis (mild derangement of liver function tests, intrahepatic cholestasis, irreversible liver injury)	3%*
Malignancy (NHL, Skin Cancer, hepatoma, colorectal cancer)	10%*
Pancreatitis	20%*
Other allergic type reactions (nausea, abdominal pain, fever, skin rashes)	12%*

* There is a considerable overlap between different forms of toxicity that occur in individual patients

Figure 2.2 Conversion of AZA to 6-MP by glutathione

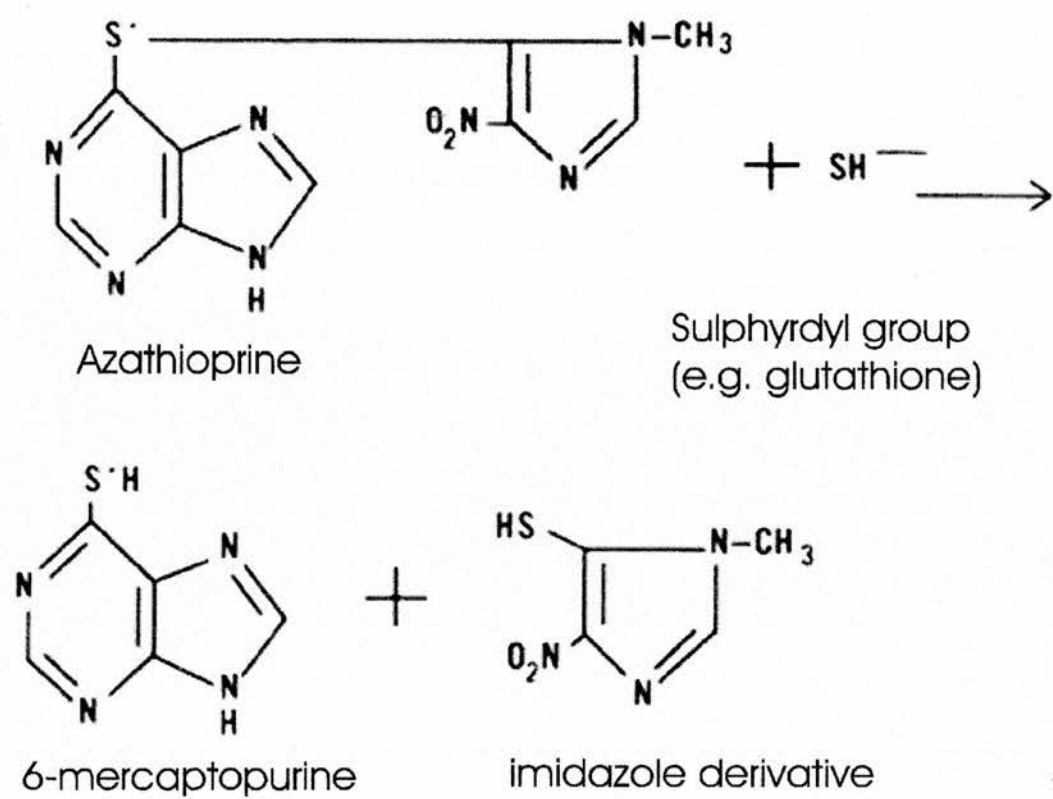
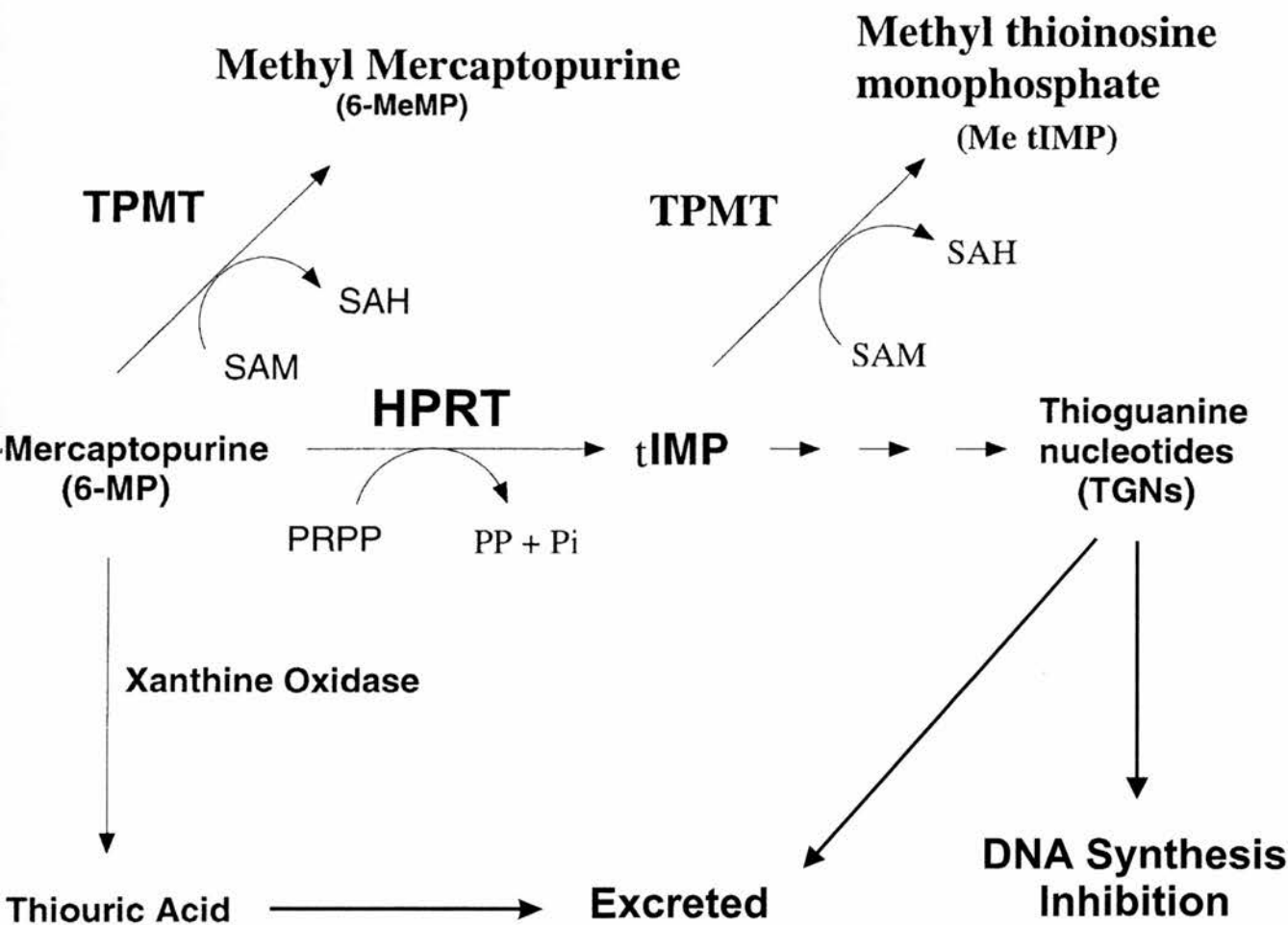


Figure 2.3. 6-Mercaptopurine metabolism.

6-MP (6-mercaptopurine), 6-MMP (6-methylmercaptopurine), HPRT (hypoxanthine phosphoribosyltransferase), 6-TGN (6-thioguanine nucleotides), SAM (S-adenosyl methionine), SAH (S-adenosyl homocysteine), PRPP (5-phosphoribosyl 1-pyrophosphate), tIMP (Inosine monophosphate)



The precise activity of the imidazole derivative has not been clearly defined. *In-vitro* evidence suggests that these imidazole derivatives possess independent immunomodulatory activity by alkylating thiol groups on T-cell surface membranes blocking antigen recognition (Crawford et al, 1996). In addition, pro-inflammatory cytokines are suppressed by high dose AZA but not 6-MP (Louis et al, 2000). Some have proposed that it is the imidazole derivative that may be responsible for some of the side effects related to AZA use (McGovern et al 2002).

The precise mechanisms of action of AZA remain to be elucidated, but suggested mechanisms are as follows: -

- 1) The release of 6-MP that acts as a purine antimetabolite.
- 2) The possible blockade of –SH groups by alkylation.
- 3) The inhibition of many pathways in nucleic acid biosynthesis, hence preventing proliferation of cells involved in the determination and amplification of the immune response.
- 4) Damage to deoxyribonucleic acid (DNA) through incorporation of purine thio-analogues and hence inhibiting de novo purine synthesis (Tidd and Paterson, 1974).

It is thought to be due to some of these mechanisms that the therapeutic effect of AZA/6-MP may take up to 3 months to become evident. These mechanisms act upon rapidly dividing cells including T and B-lymphocytes as well as gut endothelium and bone marrow elements.

2.1.2 Pharmacokinetics – Following oral administration, AZA is well absorbed from the gastrointestinal tract. Up to 30% of AZA is bound to plasma proteins and hence distribution occurs rapidly throughout the body. Following oral administration

of labelled ^{35}S -AZA, maximum plasma radioactivity occurs at 1-2 hours and then decays with a half-life of 4-6 hours. The latter reflects elimination from the plasma of AZA, rather than the half-life of AZA itself. Extensive and rapid metabolism of AZA means that only a fraction of the radioactivity measured in the plasma is comprised of unmetabolized drug. Other studies measuring AZA and 6-MP directly in the plasma following intravenous administration estimate the half-life of AZA and 6-MP to be 6-28 minutes and 38-114 minutes respectively (Dollery, 1991).

Excretion of AZA is principally in the form of thiouric acid, while the imidazole derivative component can be detected as a minor excretory product. Only a small amount of the dose of the administered AZA is excreted unmetabolized.

2.1.3 Azathioprine toxicity - The main limiting factor in AZA use are the occurrence of side effects. Between 10-15% of patients who are started on AZA will have some form of toxic reaction (Present et al. 1989). The nature of these toxic effects and the proportion of patients (out of all developing toxic effects) developing specific toxic effects is shown in table 2.1. Broadly speaking there are 2 categories of toxic reactions; namely allergic-type (e.g. pancreatitis, fever, rash, non-specific abdominal pain, nausea, rash and hepatitis) and non-allergic type (e.g. leucopenia, thrombocytopenia, infections, malignancies and some cases of hepatitis). The latter group usually occurs after months or years and appears to be dose dependent, while the former group occurs much earlier, usually within weeks.

2.2 Pharmacogenetics

2.2.1 TPMT

TPMT is a cytosolic enzyme. TPMT levels are determined by a well-documented co-dominant genetic polymorphism in Caucasian and Africo-American populations (McLeod et al. 1994; Weinshilboum and Sladek, 1980). Approximately 90% of the population has normal expression of TPMT ($TPMT^H/TPMT^H$), 10% have intermediate activity due to heterozygosity at the TPMT locus ($TPMT^H/TPMT^L$), while between 0.3 to 1% have TPMT deficiency ($TPMT^L/TPMT^L$), inherited as an autosomal recessive trait (Weinshilboum and Sladek, 1980). In the latter patients there is excessive shunting of by-products down other enzymatic pathways, leading to excesses in 6-TG's, which exert cytotoxic and immunosuppressive effects leading to bone marrow toxicity. Other adverse side effects such as pancreatitis and hepatitis are thought to be idiosyncratic reactions, unrelated to TPMT activity (Kader et al. 2000). There has been considerable interest in TPMT levels and the occurrence of AZA/6-MP toxicity. This was first investigated in children with acute lymphoblastic leukaemia treated with AZA, where it has been recognized that TPMT levels corresponded to reduced risk of relapse, but a higher risk of neutropenia (Lennard et al. 1990; Lilleyman and Lennard, 1994). In addition, TPMT genotyping has been shown to correlate well with TPMT enzymatic activity (Yates et al. 1997) and follows a trimodal population distribution as shown in figure 2.4. TPMT activity can be measured in red blood cells by a variety of methods including reverse phase high-performance liquid chromatography (HPLC) (Lennard and Singleton, 1994) and by radio-incorporation assay (Weinshilboum et al. 1978; Deininger et al. 1994). To date, there have been few studies looking at TPMT activity in IBD patients and associated

toxicity. TPMT genotyping (shown to reflect enzymatic activity) has been shown to correlate well with myelotoxicity, but otherwise does not predict hepatotoxicity or occurrence of pancreatitis.

It should be borne in mind that while TPMT measurements (genotypic and activity) clearly appear to have a useful role in identifying 'at risk' patients susceptible to myelotoxicity, other environmental factors can play a role in the development of drug toxicity such as parvovirus B19 infection (Higashida et al. 1997). Higashida et al implicated the *susceptibility* to infection with viruses such as parvovirus B19 in AZA immunosuppressed patients that may be an important factor in some patients who develop severe myelosuppression. In the case of parvovirus B19 infection, the usual resultant haematological problem is pure red cell aplasia(Higashida et al. 1997).. This means that blood count monitoring remains essential, since severe myelosuppression can occur despite normal TPMT levels. A genotype analysis of TPMT has confirmed that myelosuppression in patients on AZA is often not related to homozygous TPMT deficiency (Colombel et al 2000).

2.2.2 HPRT

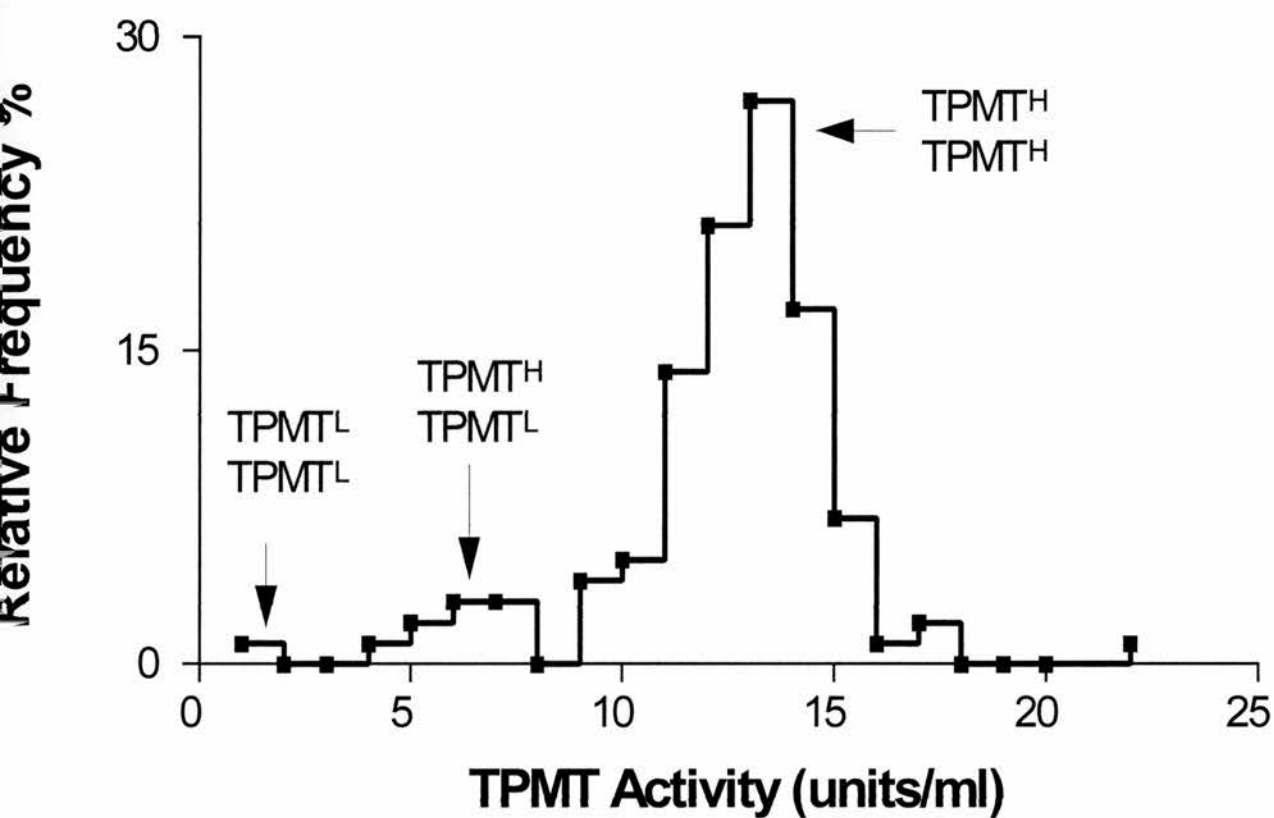
HPRT deficiency was recognized in patients with raised urate levels and predisposed to gout. In its severest form, the deficiency presents in childhood with associated neurological defects and is known as Lesch-Nyhan Syndrome. The gene that codes for HPRT is X-linked and there are several described mutations along this gene that causes a variable degree of deficiency in HPRT levels. Such is its rarity that deficiency of HPRT is probably of little clinical relevance with respect to AZA therapy in IBD patients. Such patients would be resistant to the immunosuppressive

effects of AZA therapy(Kelley et al. 1967).

Xanthine Oxidase (XO)

It is well recognized that allopurinol (which inhibits XO) interacts with AZA and can precipitate toxicity. XO catalyses the conversion of 6-MP to thiouric acid (inactive) and hence genetically low levels of XO leaves more 6-MP available for conversion into 6TG's. Xanthine oxidase deficiency is an autosomal recessive disorder, occurring in about 2% of the population, and is often picked up incidentally by the presence of hypouricaemia(Serre-Debeauvais et al. 1995). Like TPMT deficiency this condition is associated with haematotoxicity but has received only modest interest when compared to TPMT associated toxicity.

Figure 2.4 Trimodal frequency distribution of red cell TPMT activity with genotypes (TPMT^H/TPMT^H – homozygous normal TPMT; TPMT^H/TPMT^L – heterozygous intermediate TPMT; TPMT^L/TPMT^L- homozygous low TPMT)(Lennard et al. 1987)



estimated overall common odds ratio was 3.09 (95% CI, 2.45 to 3.91) in favour of AZA or 6-MP therapy and is summarized in table 2.2.

Fraser et al (Fraser et al 2002) published their retrospective 30-year experience from Oxford. They examined 192 CD patients who had received AZA for a minimum of 6 months. Complete remission (no need for concurrent steroid therapy) was achieved in 64%. The study did not comment of the ability of steroid dose reduction with AZA, and interestingly found that colonic disease was associated with a higher remission rate. Follow up in this study was for a mean of 6.9 years. Thirty nine percent of the study group discontinued AZA after 2 years of AZA achieved remission and were included in the final analysis.

- (ii) Maintenance therapy: Two studies have looked exclusively at treatment with AZA in quiescent disease(O'Donoghue et al. 1978; Rosenberg et al. 1975), while there have been several others that have included maintenance therapy as part of a multiarm study(Candy et al. 1995; Summers et al. 1979; Willoughby et al. 1971). Again, all of these exhibited varying degrees of benefit (odds ratios 1.20-4.48), and meta-analysis by Pearson et al estimated a common odds ratio of 2.27 (95% CI, 1.76 to 2.93) in favour of AZA therapy (Pearson et al. 1995), this is summarized in table 2.3. Fraser et al (Fraser et al 2002) analysed a total of 324 IBD patients (122 CD, 202 UC) in whom remission was achieved and maintained after 6 months of therapy. At 12,24,36,48 and 60 months, the proportion remaining in remission (CD and UC combined) was 0.95, 0.90, 0.69, 0.63 and 0.62 respectively. There was no difference between CD and UC groups in the rate of remission.

Table 2.2. Odds ratio of response in randomized controlled studies of azathioprine and 6-mercaptopurine for active Crohn’s disease (Pearson et al. 1995)

Study	Odds Ratio (95% CI)
Rhodes et al(Rhodes et al. 1971)	1.00 (no confidence interval, no responders)
Klein et al(Klein et al. 1974)	1.00 (0.22-4.54)
Candy (Part 1) et al(Candy et al. 1995)	1.55 (0.52-4.59)
National Cooperative Crohn’s Disease Study (Part I, Phase 1)(Summers et al. 1979)	1.57 (0.75-3.29)
Ewe et al(Ewe et al. 1993)	4.57 (1.36-15.27)
Present et al(Present et al. 1980)	10.45 (4.14-26.38)
Willoughby (Group 1) et al(Willoughby et al. 1971)	23.17 (2.57-99.9)
Common Odds Ratio	3.09 (2.45-3.91)

Table 2.3 Odds ratio of response in randomised controlled studies of azathioprine for quiescent Crohn’s disease (Pearson et al. 1995)

Study	Odds ratio (95% CI)
National Cooperative Crohn’s Disease Study, Part I, phase 2 (Summers et al. 1979)	1.73 (0.37-8.05)
National Cooperative Crohn’s Disease Study, Part II(Summers et al. 1979)	1.20 (0.60-2.41)
O’Donohogue et al(O'Donoghue et al. 1978)	2.95 (0.97-9.00)
Rosenberg et al(Rosenberg et al. 1975)	3.16 (0.57-17.62)
Willoughby et al Group 2(Willoughby et al. 1971)	4.48 (0.41-49.43)
Candy et al Part 2(Candy et al. 1995)	7.12 (2.11-23.99)
Common Odds Ratio	2.27 (1.76-2.93)

2.3.2 Ulcerative Colitis

- (i) Active disease: The use of both AZA and 6-MP in active colitis is limited due to the protracted time to maximal onset of AZA effect (approximately 3-4 months). There is no evidence that AZA plus steroids versus steroids alone increases remission rates, but there is evidence of a steroid sparing effect within this time frame(Adler and Korelitz, 1990; Kirk and Lennard-Jones 1982). In the study by Fraser et al (Fraser et al 2002), a total of 232 UC patients were given AZA following a relapse. Remission was achieved in 58% of these patients (87% in UC patients who completed a minimum of 6 months AZA). This latter figure was statistically significantly higher ($p=0.0001$) than their CD patients described before.

- (ii) Maintenance therapy: AZA therapy has been studied more extensively than 6-MP in UC. There is good evidence that AZA/6-MP allow steroid dose reduction, a conclusion found in several studies to date (AZA dose 1.5-2.5 mg/kg)(Adler and Korelitz, 1990; Caprilli et al. 1975; Jewell and Truelove 1974; Kirk and Lennard-Jones 1982; Rosenberg 1975). It should be noted that there is no clear evidence from studies to show that AZA or 6-MP are of benefit in the chronic active UC patient who is steroid resistant. Jewell et al studied such patients for 12 months and found no clear benefit(Jewell DP and Truelove SC, 1974), although uncontrolled data from others has shown beneficial effects(George et al. 1996). Further evidence can be drawn from withdrawal studies which although unable to comment on the magnitude of the effect of AZA, have shown that AZA withdrawal during remission leads to

increased relapse rates(Hawthorne et al. 1992, Fraser et al 2002). There are no controlled randomized trials that have looked exclusively at 6-MP and maintenance therapy in UC.

2.4 Current Issues

Optimal antimetabolite therapy is a minefield in current practice. This is partly due to poorly designed studies mentioned before, but more importantly a lack of specific studies looking at unanswered issues. Since there has been more extensive research into AZA and other medical conditions, conclusions have been drawn from them and extrapolated into IBD therapy – but it should be noted that some conclusions might not equally apply to IBD patients.

2.4.1 Intravenous azathioprine use: In the light of the delayed onset of action of oral AZA, there has been growing interest in the use of intravenous AZA in the hope of decreasing the time of therapeutic onset and increase the therapeutic capability of AZA in acute UC. TPMT activity status is a pre-requisite to using AZA intravenously to stop potentially fatal side effects.

The first published experience in IBD was by Sandborn et al in 1995(Sandborn et al. 1995), where CD patients were loaded with 1800 mg of AZA over 36 hours. They concluded that there was a reduced time to response in these patients. Subsequently Casson et al presented three patients with acute fulminant colitis (1UC, 1 indeterminate colitis, 1CD) who were treated with low dose intravenous AZA (3mg/kg/day) for 5-7 days(Casson et al. 1999). All three patients achieved remission on this treatment. Subsequently there have been two more publications. Firstly by Sandborn et al in 1999, who took a placebo controlled trial of intravenous loading dose of AZA (40mg/kg/36hours) in 51 patients versus 45 placebo ‘control’ patients in steroid resistant CD patients(Sandborn et al. 1999). They concluded that there was no decrease in time to response in these patients, despite achieving higher 6-TG

concentrations at 0.2 weeks and 1 week. Thereafter, 6-TG concentrations in the 2 groups were the same, hinting that oral AZA therapy achieves similar 6-TG levels to intravenous AZA. Finally, Mahadevan et al looked at acute UC in 9 patients(Mahadevan et al. 2000). Three groups of 3 were treated with 20mg/kg/36hours, 40mg/kg/36hours or 40mg/kg as three separate 8-hour infusions over 3 days. Fifty-six percent of these patients avoided colectomy, and response was seen within 4 weeks, with therapeutic 6-TG concentrations being achieved by 12 weeks. They concluded that intravenous AZA appeared to be safe and of clinical benefit in steroid refractory UC patients.

2.4.2 Azathioprine dose: Current practice involves the use of a low dose of AZA/6-MP for the first 6-8 weeks with subsequent dose adjustment to approximately 2mg/kg or even higher if tolerated by the patient. There is evidence that doses of between 1.5-2.5 mg/kg are effective in IBD, borne out in single studies and meta-analysis(Adler and Korelitz, 1990; Kirk and Lennard-Jones 1982; Rosenberg 1975). In Fraser et al's analysis (Fraser et al 2002) the mean dose of AZA was 1.65 mg/kg in those who achieved remission versus a mean of 1.64 mg/kg in those who did not achieve remission. They concluded that higher AZA dose was not predictive of the ability to achieve remission. Doses below 1.5 mg/kg probably have little clinical efficacy; with strong evidence that a dose of 1mg/kg is ineffective in CD. There are no studies that have directly compared different doses of either AZA or 6-MP to look at relapse rates or degree of steroid sparing effect. In addition there have been no trials looking at doses of more than 3mg/kg.

2.4.3 Concurrent 5-ASA use: The concomitant use of 5-ASA with AZA may have potential advantage in reducing the long-term colorectal malignancy risk associated with IBD. On the other hand there is rising concern over the possible interaction of 5-ASA compounds and their breakdown products and TPMT – the enzyme used in the handling of both AZA and 6-MP(Woodson et al. 1983). *In-vitro* studies have suggested that 4-ASA, 5-ASA and sulphasalazine all inhibit TPMT activity to differing degrees (Lewis et al. 1997; Lowry et al. 1999; Szumlanski and Weinshilboum, 1995). While the efficacy of long-term remission maintenance of concurrent 5-ASA use with AZA versus AZA alone remains unstudied to date, the long term clinical impact on colorectal malignancy risk remains unanswered. Until there are studies to address this issue, it would seem sensible to add in AZA/6-MP therapy with concurrent 5-ASA treatment where indicated being aware of a potential drug interaction. Further prospective studies are now required.

2.4.4 Neutropenia: It is a matter of debate whether neutropenia is a prerequisite for optimal AZA therapy. In current clinical practice, AZA dose is not titrated against neutropenia, unless dangerous levels of neutropenia are seen.

There has been interest in the role of AZA/6-MP induced leucopenia in accomplishing disease remission (Colonna and Korelitz 1994). The large retrospective study by Fraser et al (Fraser et al 2002) found a lower nadir WCC and lower nadir neutrophil count was predictive of achieving remission in IBD patients, and also suggested that a lower nadir WCC helped keep these patients in remission for longer. In addition there have been anecdotal reports of prolonged remission in patients who have sustained pancytopenia during AZA therapy(Burke et al. 1989). More recently, it has been the significance of white cell differential in leucopenia patients which has received

interest (Korelitz et al. 1997). It has been proposed that it is the preferential suppression of neutrophils during leucopenia that effects remission induction with AZA/6-MP. The lack of studies concerning this area of treatment strategy means that neutrophil count should not currently be used as an end point to dose titration of AZA or 6-MP.

2.4.5 Mean Corpuscular Volume: It has long been recognized that AZA/6-MP causes blood count abnormalities such as leucopenia, thrombocytopenia and raised MCV. The latter parameter is not a sign of toxicity. The mechanism by which MCV increases is thought to be related to interference of cell proliferation in blood precursors committed to erythropoiesis, with subsequent adaptive changes leading to megaloblastic erythropoiesis (Williams et al. 1978). The rise in MCV following administration of AZA/6-MP is not necessarily inevitable, nor does the level that the MCV rise correlates with drug dosage (Nicholls and Davidson, 1979).

Mean corpuscular volume changes have been studied extensively in renal transplant patients, but only one study has been published in IBD patients. Fraser et al (Fraser et al 2002) found that a higher mean MCV was a predictor of achieving remission in IBD patients (mean 93.3 in remission achieved versus 88.8 in remission not achieved, $p=0.0001$).

Studies looking at MCV changes (δ MCV) during therapy have been shown to be a reasonable indirect estimation of 6-TG levels, but advocated a prospective study before this parameter is utilized in clinical practice (Decaux et al. 1999). 6TG levels measured directly have been shown to correlate well with actual bioavailability of oral AZA/6-MP and hence ability to retain disease remission during therapy.

2.4.6 6-TG monitoring: There has been increasing interest in the use monitoring 6-MP metabolite levels as a more accurate method of assuring drug response, and compliance. Erythrocyte 6TG levels represent an indirect measure of bone marrow uptake and hence degree of immunosuppression. Red cell 6-TG levels can be measured by a modification of a reversed phase HPLC (high performance liquid chromatography) method initially used to measure TPMT levels(Lennard and Singleton, 1994). 6-TG levels have been shown to exhibit a significant inverse correlation with the HBI (Harvey-Bradshaw Index) in CD(Cuffari et al. 1996). Thus measurements of 6-TG levels are useful in assessing both drug compliance, responsiveness to treatment while preventing under and over-dosing.

2.4.7 Pregnancy and fertility issues: Clinicians are not under any form of compulsion to report the outcome of administration of drugs during pregnancy to a central monitoring authority. Therefore, data concerning both pregnancy and fertility in patients taking AZA are confined to those voluntarily reports submitted for publication and those reported to the pharmaceutical company. Initial published literature was in renal transplant patients, following which, smaller and less detailed reports have been published in other medical conditions such as rheumatoid arthritis, SLE, renal transplantation, autoimmune hepatitis and IBD(Alstead et al. 1990; O'Donnell et al. 1985; Ramsey-Goldman et al. 1993; Steven et al. 1979). In the latter disease group there is a clear paucity of published data, although the reported risk of foetal abnormalities from such patients taking AZA is approximately 2%. There is no doubt that AZA crosses the placenta and is expressed in breast milk in mothers taking AZA. However, the plasma concentrations are small representing up

to 14% of the orally administered dose, and thus it appears unlikely that such metabolite concentrations will have a clinically significant effect on the foetus (Rosenkrantz et al. 1967). Cytogenetic analysis has detected chromosomal aberrations in offspring of patients with CD, but long term follow up has not revealed further problems in these infants (Willoughby et al. 1971). Immunological abnormalities have also been reported in the newborn's of these patients (Golby, 1970; Williamson and Karp, 1981), constituting lymphopenia, thymic hypoplasia and bone marrow hypoplasia, but all of these have subsequently resolved later in infancy. Unfortunately, there is a lack of reports of detailed immunological assessments in these patients. Table 2.4 summarizes the relative percentage occurrence of reported foetal abnormalities in patients taking AZA.

Overall, good control of the patient's IBD is much more important for the well being of the foetus and pregnancy. It should be remembered that patients with CD have higher rates of spontaneous abortion and stillbirths, and that controlling disease activity may reduce these risks (Alstead et al. 1990).

There are a lack of publications that have looked at male fertility in IBD patients taking AZA. Spermatogenesis has been studied in renal transplant patients and shown to be similar to healthy controls (Evans et al. 1975), other small reports in renal transplant patients revealed decreased sperm counts but the counts remained within the normal range (Handelsman et al. 1984). In a study reported after the commencement of our own work, male fertility on AZA appeared to be normal in IBD patients (Dejaco et al 2001), despite earlier concerns in the rheumatological literature (Janssen et al 2000).

2.4.8 Neoplasia Risk: It has been well recognized that the risk of various malignant disorders is higher in transplant recipients receiving immunosuppressive therapy than in the general population. In particular prospective studies have shown an excess of non-Hodgkin lymphoma (NHL), squamous cell carcinoma and hepatoma (Hoover and Fraumeni, Jr., 1973; Kinlen et al. 1979; Kinlen and Hoover, 1979). In addition, rheumatoid arthritis patients receiving AZA have been shown to exhibit excessive frequencies of these malignancies beyond that associated with rheumatoid disease (Silman et al. 1988). Studies in IBD patients have been sparse. One IBD study was by Connell et al in 1994 who looked at 755 IBD patients taking AZA (2mg/kg) studied over a 29 year period (Connell et al. 1994). They concluded that while colorectal carcinoma occurred in excess (something observed in IBD patients anyway), there were no cases of NHL, although the study's power was relatively low. Another study by Present et al looked at 396 IBD patients, treated with 6-MP for a mean period of 5.4 years. They described 12 cases of malignancy, 1 of which was NHL (Present et al. 1989). Finally, a large analysis of 782 IBD patients by Farrell et al concluded that although there was an increased risk of non-Hodgkin's lymphoma, but the overall risk was low (Farrell et al. 2000). Clearly, as long as patients are informed of the potential and seemingly small risks of AZA therapy, it seems sensible that AZA should be used considering the morbidity associated with other long-term medical therapies and risks from surgery. Table 2.5 summarizes published relative risks of developing malignancy in patients receiving AZA.

2.4.9 Duration of therapy: One of the most frequently asked questions by patients taking AZA is how long does treatment last. There have now been several long-term studies looking at both AZA and 6-MP use in both CD and UC. Probably the most

quoted paper is that from Bouhnik et al that first addressed this question(Bouhnik et al. 1996). They looked at 157 patients over a 6-year period. Relapse rates were examined in patients on AZA/6-MP compared with those whose AZA/6-MP therapy was discontinued. They concluded that beyond 4 years of therapy, relapse rates were the same for both groups. It should be noted that at this time point, the two groups were small and hence may not have had the power to detect a significant difference in relapse rates. A smaller study involving 105 UC patients came to a different conclusion (George et al. 1996). Duration of follow up was approximately 5 years, but relapse in the withdrawal group was very small (n=15) making conclusions somewhat difficult to interpret. More recently, Kim et al looked at 6-MP therapy in 120 CD patients with a follow up period of over 8 years (Kim et al. 1999). Again study design was similar, but final conclusions from this study found statistical significance between 6-MP and withdrawal group. Their conclusions advocated the indefinite use of 6-MP therapy once remission was achieved, although admitted that until there is a placebo-controlled withdrawal trial, optimal duration of therapy is unlikely to be known. Fraser et al (Fraser et al 2002) also examined IBD patient relapse after cessation of AZA therapy. Duration of AZA before cessation was not predictive of the duration of remission, and concluded that from their data there was no evidence to support the concept of no benefit after 4 years as proposed by Bouhnik et al. Indeed at 5 years into AZA therapy some 62% of patients remained in remission. A recent randomised controlled trial by the GETAID group of AZA withdrawal after at least 42 months of therapy in CD patients in good remission showed that AZA withdrawal was associated with a higher frequency of relapses. (Lemann et al 2002). Clearly there appears to be good evidence for use up to 4-5 years, and if the drug is well tolerated longer therapy may well be indicated. In the authors experience it is

often the patient that will request discontinuation of therapy after prolonged remission and therefore it is important that patients are aware that evidence suggests higher relapse rates once therapy is withdrawn even after several years.

2.4.10 Azathioprine or 6-Mercaptopurine therapy? Although the side effect profiles of both AZA and 6-MP are very similar, there is evidence of different bioavailabilities of these two drugs, and between branded AZA and generic AZA (Cuffari et al. 2000). This has further implications with regard to optimizing drug treatment with 6-TG monitoring. In addition, there are small series of patients who have been re-challenged with 6-MP after being intolerant to AZA. In a group of 11 patients, 54% (6) were able to tolerate 6-MP, hinting that 6-MP may have benefits over AZA for initial therapy (Bowen and Selby 2000). McGovern et al (McGovern et al 2002) has also suggested that it may be the imidazole moiety that is released during AZA breakdown that may be responsible for the intolerance of AZA and the tolerance of 6-MP.

2.5 Conclusion

In summary, antimetabolite therapy has proven itself an extremely valuable and efficacious drug that now forms an invaluable option to the gastroenterologist in helping treat steroid refractory/dependent IBD. Although there may well be a great number of small and poorly designed studies, experience with time has given us increasing understanding in helping to develop ever more optimal immunosuppressive therapy in IBD patients. Its long-term safety is now generally well accepted with appropriate monitoring and with more studies, further information will help us refine antimetabolite use in the future.

Table 2.4 Percentage occurrence of foetal abnormalities reported in the

literature (Alstead et al. 1990; O'Donnell et al. 1985; Ramsey-Goldman et al. 1993;

Steven et al. 1979; Golby 1970)

Foetal Complication	% Occurrence
Spontaneous abortion	23
Haematological abnormalities	17
Foetal/Perinatal death	15
Cardiac abnormalities	11
Skeletal abnormalities	6
Perinatal Infection	9
Neural tube defects	4
Other abnormalities	15

Table 2.5 Relative risk of malignancy associated with AZA therapy (including non-IBD patients treated with AZA)(Fraser et al 2002; Farrell et al 2000, Connell et al 1994; Gaya et al 1995; Kinlen et al 1985; Lewis et al 2001)

Malignancy type	Relative Risk Range
Colorectal Cancer	4.3-30.8*
Stomach	1.0-2.1*
Lung	0.75-1.0
Cervix	1.0-4.0
Non-Hodgkin’s Lymphoma	1.0-33.8**
Skin	1-20**

*No significant difference found when compared against disease matched controls

** Relative risk increased with duration of AZA therapy

Chapter. 3. Is neutropenia in inflammatory bowel disease patients treated with azathioprine desirable?

3.1 Introduction

AZA use in IBD has been now widely accepted as an invaluable second line immunosuppressive agent in the treatment of steroid refractory or steroid dependent disease (Marion and Present 1997; Pearson et al. 1995). The optimal therapy regimen for AZA use continues to be defined as experience with its use in IBD grows.

AZA is usually introduced at a low dose and titrated up to an arbitrary dose of approximately 2mg/kg, although higher doses are generally used in the United States. The mean time to onset of effect of AZA is about 3 months, and it is generally accepted that a dose of between 2-2.5 mg/kg is efficacious. AZA is broken down to 6-MP and then on to active 6-thioguanine molecules by the enzyme thiopurine-methyl-transferase (TPMT). TPMT is genetically determined, and inherited in a co-dominant fashion (Dubinsky et al. 2000). Genetically low levels of TPMT have shown to correlate well with the occurrence of some AZA induced side-effects, in particular, the development of neutropenia (Colombel et al. 2000).

Both macrocytosis and lymphopenia are well recognized haematological effects of AZA therapy and their relevance to achieving or maintaining remission remains uncertain (Colonna and Korelitz 1994; Decaux et al. 1999; Colonna and Korelitz 1994; Decaux et al. 1999). The dangers of leukopenia and, perhaps more importantly neutropenia have long been recognized, necessitating regular blood count monitoring of patients taking AZA.

More recently, there has been interest in the potential therapeutic value of ‘safe’ levels of neutropenia and leukopenia in achieving and maintaining remission (Colonna and Korelitz 1994; Korelitz et al. 1997). Anecdotal reports have suggested that these may be now desirable end points to AZA therapy (Burke et al. 1989). However this remains unanswered and in current clinical practice, AZA dose is not titrated against neutropenia, unless dangerous levels of neutropenia are seen.

3.2 Patients & Methods

The hypothesis was that there is no difference in remission maintenance in IBD patients treated with AZA who achieve a neutropenic neutrophil count compared to those who do not.

A database was constructed containing information on IBD patients maintained on AZA (n=203; UC=94; CD=109; median age 39 years, range 15-82 years, 88 male:115 female). For the purpose of this study, patients were excluded if they were not maintained on AZA for a minimum of 6 months or if their treatment was stopped due to non-haematological side effects, or if their disease remained active despite AZA therapy. This left a total of 173 IBD patients (UC=77;CD=96, median age 39 years, range 16-81 years; 76 male, 97 female) who had been maintained in remission on AZA. Median duration of follow up was 4.0 years (range 0.6 – 21 years). Other information that was collected included the lowest neutrophil count ever recorded during therapy, the lowest neutrophil count within the first 4 months of therapy, anatomical disease distribution, AZA dose, patients weight at induction of therapy and smoking status. Relapse was defined as necessity for surgery, or clinical recurrence (as assessed by an experienced clinician based on clinical features of diarrhoea, abdominal pain, general well being, weight loss and inflammatory parameters – haemoglobin, ESR, CRP, white cell count and platelets). Requirement for surgery for purely mechanical obstruction, without abnormal inflammatory parameters was not considered a relapse of active disease. Endoscopic findings complemented clinical features and blood parameters, but were not used solely to determine relapse. Relapse rates per year of follow up were compared with the lowest degree of neutropenia

achieved during AZA therapy. A neutrophil count of $\leq 2.5 \times 10^9$ was used to define neutropenic patients. All cases were examined by one investigator (SC). There were 44 patients who had been neutropenic (mean neutrophil count 1.9; SD 0.34) while taking AZA, with 129 patients not achieving any neutropenia (mean neutrophil count 3.7; SD 0.9). Doses of AZA were similar in both groups of patients (neutropenic 1.8mg/kg, SD 0.41; UC 1.9 mg/kg, SD 0.42; CD 1.75 mg/kg, SD 0.43; non-neutropenic 1.9mg/kg, SD 0.47; UC 1.85 mg/kg, SD 0.48; CD 1.95 mg/kg, SD 0.45). Also comparable were mean duration of follow up, duration of therapy, disease anatomy, concurrent use of steroids and use of 5-ASA drugs. These results are summarized in table 3.1.

3.2.1 Statistical analysis - Statistical analysis was by non-parametric Mann-Whitney test for comparing the two groups. Relapse rates were calculated using Kaplan Meier survival curves and compared using log rank test analysis.

Table 3.1: Group characteristics (disease distribution, AZA dose, duration of treatment and neutrophil counts)

Group	N=	CD:UC	CD disease distribution	UC disease distribution	Mean AZA Rx (years)	Mean Age (years)	M:F	Mean AZA dose (mg/kg)	Mean lowest neutrophil count
Non- neutropenic ($>2.5 \times 10^9$)	129	79:50	TI=15 Colitis=50 Ileocolonic=14	Pan colitis =13 Left sided=20 Proctitis=17	4.8	38	49:80	1.9	3.2
Neutropenic ($\leq 2.5 \times 10^9$)	44	16:28	TI=3 Colitis=10 Ileocolonic=3	Pan colitis=7 Left sided=14 Proctitis=7	5.0	41	17:27	1.8	1.9

3.3 Results

A total of 173 IBD patients were stable on AZA for a minimum of 6 months. Median duration of therapy was 4.0 years (range 0.6-21 years). Forty-four patients (25 %) became neutropenic (lowest neutrophil achieved during therapy of $\leq 2.5 \times 10^9$) while taking AZA of which 4 patients had stopped treatment due to severe life threatening neutropenia (2 CD, 2 UC), and were included in the final analysis. The two Crohn's patients suffered from severe sepsis. One was from a urinary tract infection (lowest neutrophil count 0.5×10^9) and resolved with intravenous antibiotics. The other patient developed neutropenic sepsis necessitating the use of Granulocyte Colony Stimulating Factor (GCSF, Filgrastim, Roche, Welwyn Garden City) (lowest neutrophil count 0.2×10^9), intensive care admission for 10 days and haemodialysis, but made a full recovery. One of the UC patients developed severe pancytopenia and subsequent Adult Respiratory Distress Syndrome (ARDS - lowest neutrophil count 0.8×10^9), necessitating intensive care treatment for 5 days, but made a full recovery. The other UC patient developed no complications (lowest neutrophil count 0.8×10^9) and their blood count returned to normal within a week of stopping AZA.

For all IBD patients (M=66, F=107), mean relapse rates in the neutropenic group (mean AZA dose 1.80 mg/kg, SD 0.41, M=17, F=27) were 0.28/year follow up (SD=0.43) compared with 0.19/year follow up (SD=0.37) for the non-neutropenic group (mean AZA dose 1.90 mg/kg, SD 0.47, M=49, F=80); this was not statistically significant ($p=0.37$ Mann-Whitney test). Figure 3.1 shows cumulative survival curves

for IBD patients with no statistical difference between neutropenic and non-neutropenic groups by log rank analysis ($\chi^2=1.49$, $p=NS$).

For the UC patients (M=22:F=56), the neutropenia group (mean AZA dose 1.90 mg/kg, SD 0.42, M=11: F=17) had a mean relapse rate of 0.30/year follow up (SD=0.48) compared with 0.18/year follow up for the non-neutropenic group (mean AZA dose 1.85 mg/kg, SD 0.48, M=11:F=39; $p=0.63$ MWT). For CD patients (M=44:F=51), the neutropenic patients (mean AZA dose 1.75 mg/kg, SD 0.43, M=6:F=10) had a mean relapse rate of 0.20/ year follow up (SD=0.32) compared with 0.23 /year (SD=0.44) follow up in the non-neutropenic group (mean AZA dose 1.95 mg/kg, SD 0.45, M=38:F=41, $p=0.41$ MWT). Figure 3.2 shows cumulative remission percentage in UC and Figure 3.3 CD using Kaplan-Meier survival curves. There is no statistical difference between neutropenic and non-neutropenic groups ($\chi^2=0.76$ for UC, $\chi^2=0.33$ for CD, $p=NS$). These results are summarized in table 3.2.

In addition, univariate and multivariate analysis were performed in smoking and non-smoking groups. These were not statistically significant in any group.

Figure 3.1: Kaplan-Meier survival curve for IBD patients

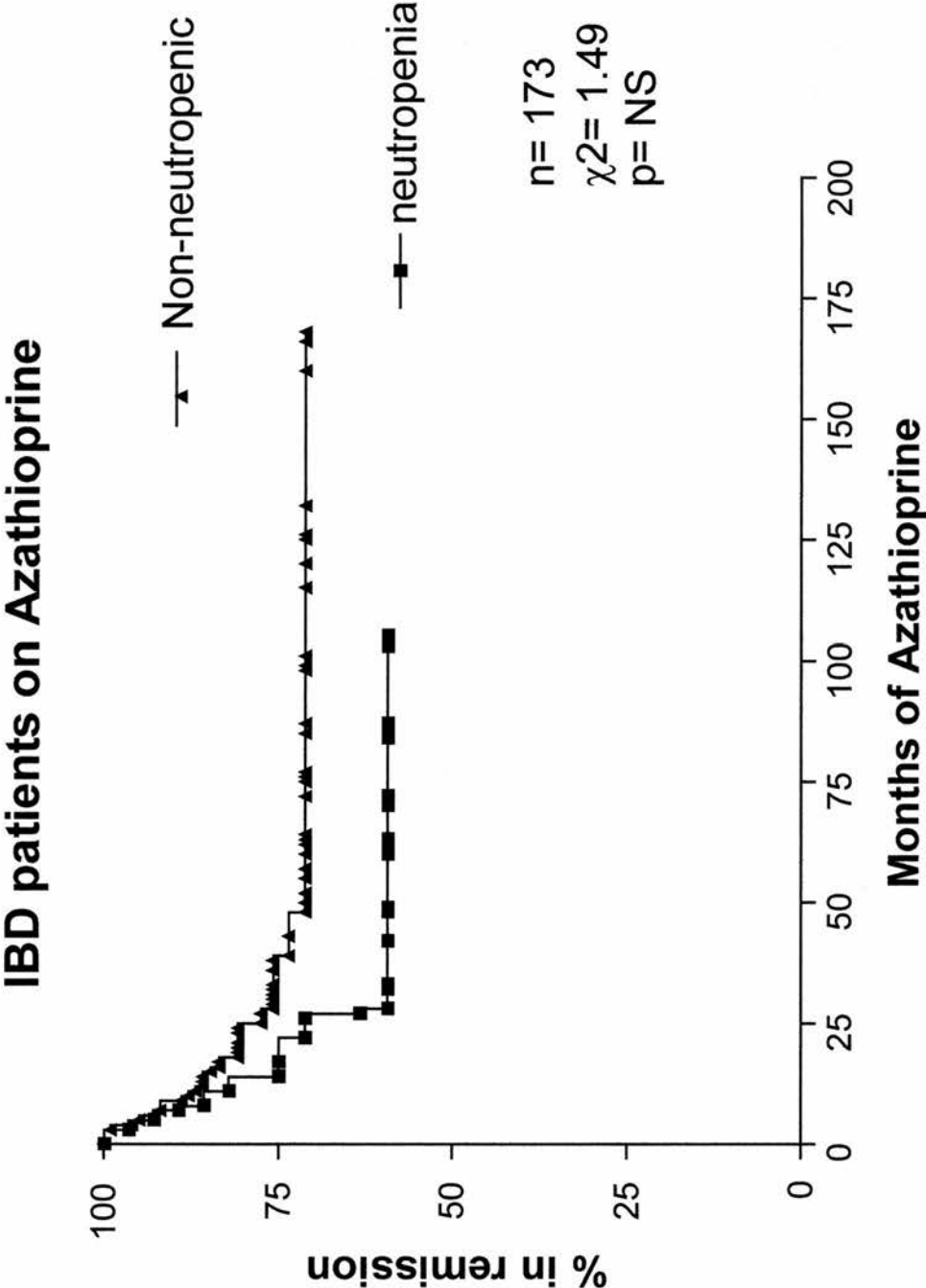


Figure 3.2: Kaplan-Meier survival curve for UC patients

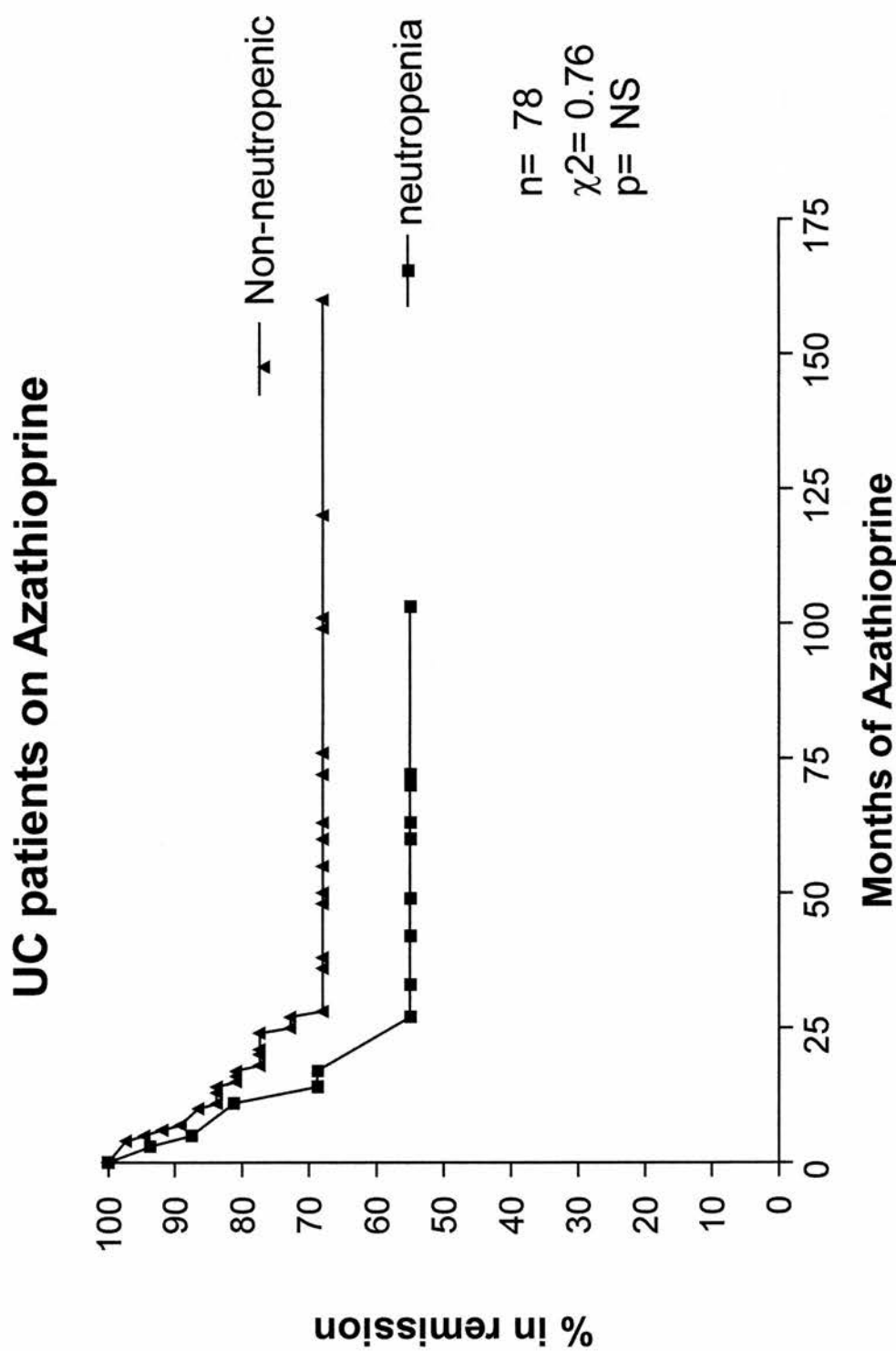


Figure 3.3: Kaplan-Meier survival curve for Crohn's disease patients

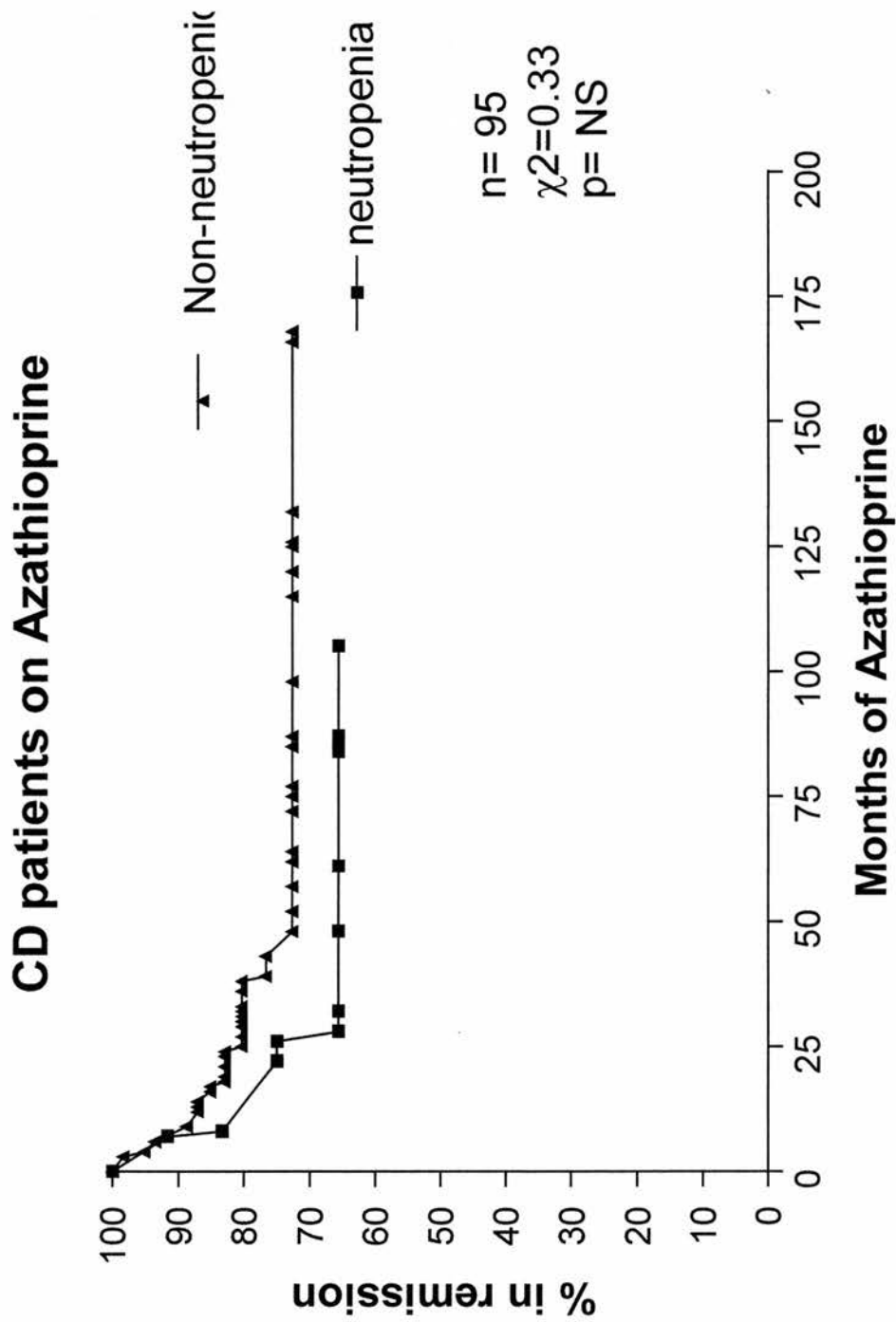


Table 3.2: Relapse rates for IBD, Ulcerative colitis, and Crohn’s disease

Group Analysis	N=	Mean relapse rate/yr follow up	Range (relapse rate) / year follow up	SD	Mann Whitney Test (non-neutropenic vs. neutropenic)	Mean AZA dose (mg/kg)
UC (non-neutropenic)	50	0.18	0-1.0	0.23	P=0.41	1.85
UC (neutropenic)	28	0.30	0-2.0	0.48		1.90
CD (non-neutropenic)	79	0.23	0-2.6	0.44	P=0.48	1.95
CD (neutropenic)	16	0.20	0-1.0	0.32		1.75
IBD (non-neutropenic)	129	0.19	0-2.6	0.37	P=0.37	1.90
IBD (neutropenic)	44	0.28	0-2.0	0.43		1.80

3.4 Discussion

From this cohort of 173 patients remission maintenance is not statistically different in neutropenic versus non-neutropenic patients. If anything, the survival curves suggest that time to first relapse is shorter for patients who achieved neutropenia (neutrophil counts $\leq 2.5 \times 10^9$). Further analyses were performed using $\leq 2 \times 10^9$ as the cut-off for the neutropenic groups (data not shown) and again no significant difference in relapse rates were found. In addition we analysed the lowest neutrophil counts within the first 4 months of therapy (data not shown). This correlated well with the nadir neutrophil count (Pearson correlation coefficient $r = 0.7$, $P < 0.005$); again supporting that neutropenia is not purely a sporadic phenomenon. In view of the fact that neutropenia is not a sporadic phenomenon, we have not used the mean of neutrophil counts, but the lowest neutrophil count in our study. The lowest neutrophil count reflected in general the pattern of neutrophil counts closely.

AZA immunosuppression is probably mediated through its effects on lymphocytes, although there appears to be preferential suppression of neutrophils when leukopenia occurs (Korelitz et al. 1997). It has been suggested that AZA's anti-inflammatory action may be mediated through its effects on neutrophils, and it has been shown that neutrophil migration into the lamina propria occurs during clinical relapse in IBD (Teahon and Bjarnason 1993). However, the complex mechanism by which AZA affects many cell lines means that it is difficult to implicate these lines in the pathogenesis of IBD. Indeed. An anecdotal report has described prolonged remission of in excess of 4 years following AZA induced pancytopenia (Burke et al. 1989). This report compares well with our own experience, where 3 of the 4 patients that

developed severe neutropenia have remained in remission up to 21 months following that episode. More interestingly, the 3 patients achieving remission all developed sepsis necessitating hospital admission for treatment.

Probably the best documented study looking at the role of leukopenia was by Colonna et al (Colonna and Korelitz 1994), whom studied 98 CD patients retrospectively. They concluded that patients achieving leukopenia were more successful in reducing their CCFA-IOIBD (Crohn's and colitis Foundation of America/International organization of inflammatory bowel disease) index and in maintaining or inducing clinical remission. In addition these patients were more able to reduce their corticosteroid dose. However, this study had a relatively short period of follow-up of about 18 months, and did not look specifically at neutropenia.

Our study involves a large number of patients over a long period of follow up of up to 21 years. Despite this being a retrospective analysis, and hence subject to type II errors, this study has an 80% power to detect a 30% difference between the two groups. Thus it could be argued that a smaller difference between the two groups may not have clear implications considering the potential risks of neutropenia. Since neutropenia can occur at any time during therapy and hence relatively unpredictable, a randomised study may prove difficult and possibly hazardous to perform. However the retrospective and observational nature of our study needs to be considered in interpreting results which could be biased by patient selection and only a randomised study with AZA metabolite and careful neutrophil monitoring can conclusively answer the question.

In conclusion, these data do not support the hypothesis that patients who achieve a nadir neutrophil count of $\leq 2.5 \times 10^9$ while taking AZA maintain disease remission for longer. A prospective, randomized trial of AZA induced neutropenia is required to ascertain whether neutropenia is a desirable end-point in IBD patients.

Chapter 4. 5-Aminosalicylate drug use in conjunction with Azathioprine therapy in inflammatory bowel disease patients

4.1 Introduction

AZA therapy is an extremely valuable second line therapy in IBD patients who are refractory to or dependent on steroid therapy. Optimal drug therapy in patients on AZA still remains unanswered, with a considerable percentage of patients remaining on a 5-aminosalicylate (5-ASA) derivative as well as AZA. Some may also require low dose corticosteroid therapy.

The concomitant use of 5-ASA with AZA may have potential advantage in reducing the long-term colorectal malignancy risk associated with IBD. On the other hand there is rising concern over the possible interaction of 5-ASA compounds and their breakdown products and TPMT – the enzyme used in the handling of both AZA and 6-MP. *In-vitro* studies have suggested that 4-ASA, 5-ASA and sulphasalazine all inhibit TPMT activity to differing degrees, but as yet there has been no studies looking at the clinical impact and side effect profiles in patients who take AZA and concurrent 5-ASA drugs.

It is not known whether concomitant 5-ASA therapy is required in patients maintained in remission with azathioprine. It is possible that 5-ASA therapy may be of additional

benefit in maintaining remission in UC but 5-ASA is unlikely to have such a role in CD. In a retrospective study, we compared relapse rates and AZA discontinuation rates in both UC and CD patients maintained in remission on AZA alone with that in patients' maintained on AZA and a 5-ASA drug.

4.2 Patients & Methods

The hypothesis was there is no difference in remission maintenance in IBD patients on concurrent 5ASA+AZA therapy versus IBD patients on AZA therapy alone.

From our database of IBD patients maintained on AZA (n=203;UC=94;CD=109; median age 39 years, range 15-82 years, 88 Male:115 Female), we retrospectively examined relapse rates per year of follow up (i.e. total number of relapses divided by duration of follow up). All case notes were examined by one investigator (SC).

Relapse was defined as a requirement for surgery or documented symptoms consistent with a relapse necessitating rescue medication such as corticosteroid therapy. Thirty-four patients (16%) had stopped AZA due to side effects (dangerous blood dyscrasias, pancreatitis, nausea, abdominal pain, skin rashes or myalgia). Seventeen patients developed side effects late into AZA therapy before discontinuation while the remaining other 17 patients encountered side effects within the first 6 months of treatment and were never stabilized on AZA – this latter group were excluded from further analysis. This left a total of 186 patients (median age=39 years, range 15-82 years, 75 Male:111 Female) who were stable on therapy for a minimum of 6 months and in remission. Median duration of follow up was 3.8 years (range 0.6-15.5 years). Information was also examined on the 34 patients who had to discontinue AZA due to adverse side effects, and assessed whether the patients were on a 5-ASA at the time of discontinuation.

One hundred and three (55%) out of 186 patients were taking AZA for a minimum of 6 months were also on concomitant 5-ASA drug (mean AZA dose for

UC=1.75mg/kg, range 0.8-3.5mg/kg; CD=1.70mg/kg, range 0.8-2.7 mg/kg), while 83 patients were taking AZA alone (mean AZA dose for UC=1.85mg/kg, range 1.0-2.7 mg/kg; CD=1.8mg/kg, range 0.6-3.0 mg/kg). Median 5-ASA doses were 2.4g mesalazine/day (range 0.8-3.2g/day n=71) (Asacol and Pentasa), 1.5g sulphasalazine/day (range 1-4g/day, n=30), while 2 patients were taking 7.5g balsalazide/day and 2 patients 1.75g olsalazine/day. The mean dose of AZA (AZA), duration of therapy, frequency of use of low dose corticosteroid in the two groups, mean neutrophil counts and disease anatomy were comparable. These characteristics are summarized in table 4.1.

4.2.1 Statistical analysis - Statistical analysis was by Students 't' test for comparing continuous variables between the two groups, AZA alone and AZA + 5-ASA in UC and CD separately. Categorical data were compared using the chi-squared test with Yates' correction. Kaplan Meier Survival Curves were constructed using time to first relapse, and compared between the two groups using log rank test.

4.3 Results

186 IBD patients were stable on AZA for a minimum of 6 months. The mean duration of therapy was 3.8 years (SD=3.0). One hundred and three patients (55%) were taking concurrent 5-ASA drugs.

Analyses were performed for both UC and CD patient groups separately. In the UC group (n=82), patients taking AZA + 5-ASA (n=55) had a mean relapse rate of 0.21/year follow up (SD=0.31) compared with the AZA alone (n=27) group with a mean relapse rate of 0.19/year of follow up (SD=0.28) (p=0.69; Student 't'-test). In the CD group (n=104), AZA + 5-ASA patients (n=48) relapse rates were 0.27/yr follow up (SD=1.0) compared with the AZA alone group (n=56) relapse rate 0.30/yr follow up (SD=0.8), (p=0.97; Student 't'-test). Figure 4.1 shows remission percentage based upon time to first relapse for UC patients, Figure 4.2 shows the same analysis in CD patients. There was no statistical difference between those on AZA alone and on AZA + 5-ASA in either disease by log rank test ($\chi^2=0.06$ for UC and 0.01 for CD respectively, p=NS). These results are summarized in table 4.2

5-ASA therapy (n=15) was no more frequent in those patients who had to discontinue AZA therapy (n=34) due to side effects (Number discontinued in AZA alone group versus number discontinued in AZA +5-ASA group: $\chi^2=0.4$, p=NS). In addition, comparing the 17 patients who discontinued AZA before stabilization (i.e. before 6 months) against those whom discontinued after 6 months (n=17) showed no significant differences in side effect types or mean dose of AZA. This is summarized in table 4.3.

Finally, there were no cases of malignancy in this group, although one patient with CD developed Waldenstroms macroglobulinaemia after 7 years AZA and mesalazine therapy, and two patients with UC developed low-grade dysplasia, one after 5.8 years of treatment with AZA and sulphasalazine and the other 3 years of treatment with AZA and mesalazine.

Figure 4.1 Remission percentages based upon time to first relapse for ulcerative colitis patients

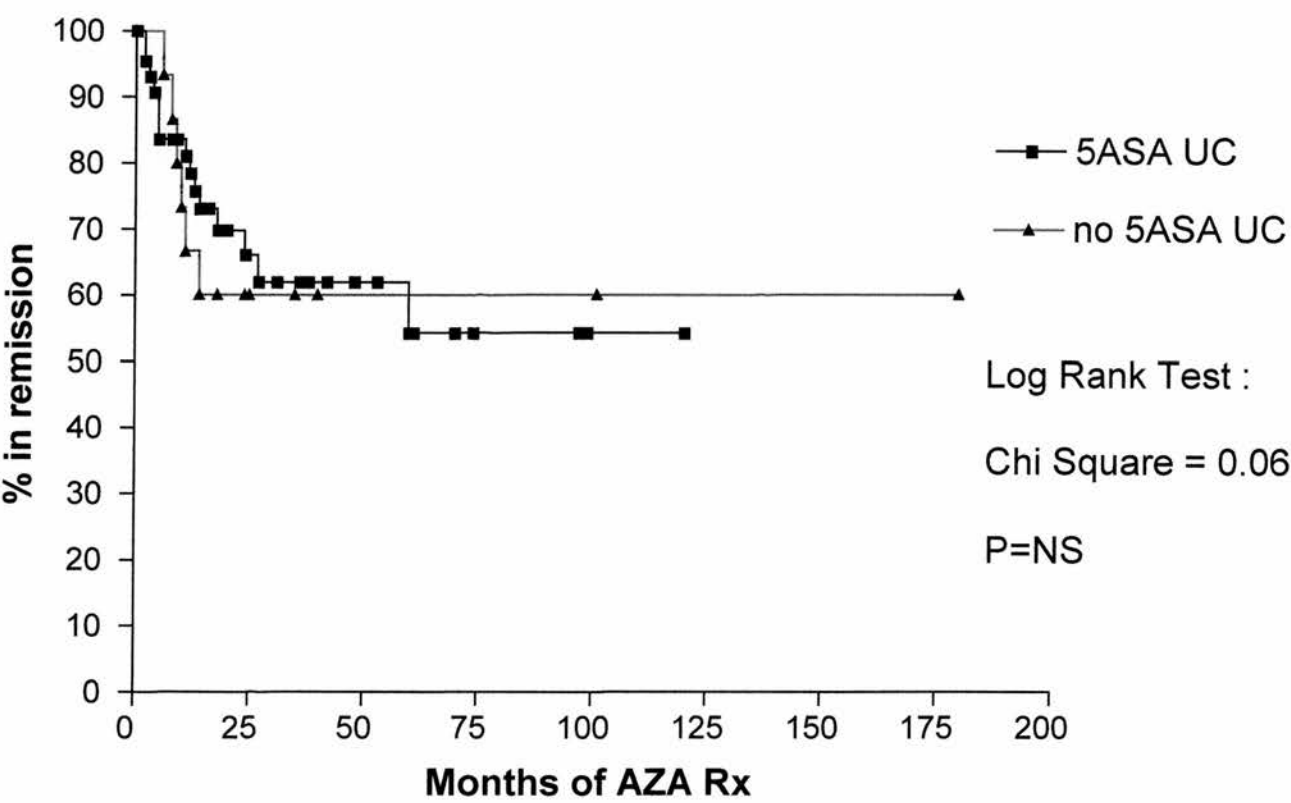


Figure 4.2 Remission percentage based upon time to first relapse
for Crohn’s disease patients

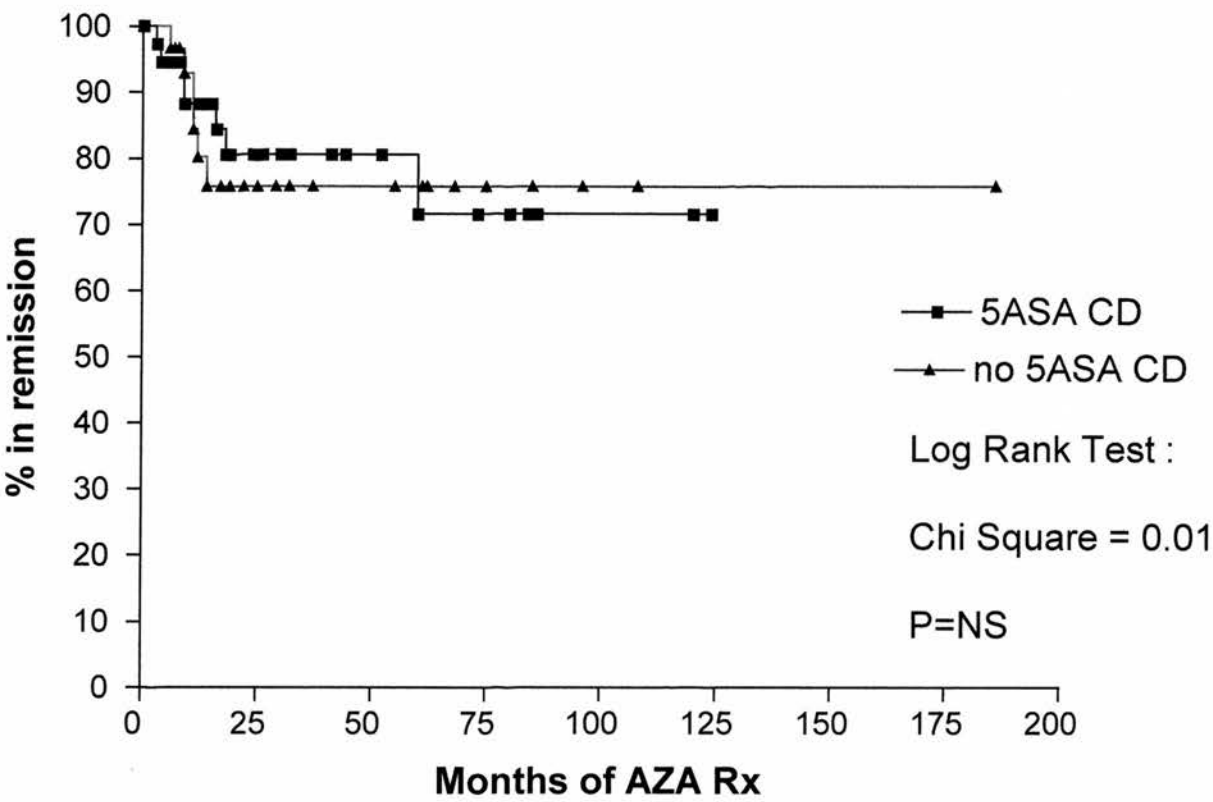


Table 4.1 Patient characteristics

(Disease distribution, steroid use, azathioprine therapy duration and neutrophil counts)

Group	N=	Disease distribution	Mean duration of AZA Rx (years)	Mean Age (years)	M:F	Mean AZA dose (mg/kg) [RANGE]	Patients taking low dose corticosteroid	Mean Neutrophil Count
AZA+5ASA (UC)	55	Pan colitis = 19 Left sided = 18 Proctitis = 18	4.7	45	30:25	1.75 [0.8-3.5]	11	3.8
AZA+5ASA (CD)	48	TI = 8 Colitis = 31 Ileocolonic = 9	4.0	40	15:33	1.70 [0.8-2.7]	10	4.3
AZA alone (UC)	27	Pan colitis = 11 Left sided = 8 Proctitis = 8	4.3	43	14:13	1.85 [1.0-2.7]	4	4.7
AZA alone (CD)	56	TI = 15 Colitis = 26 Ileocolonic = 15	4.6	38	30:26	1.80 [0.6-3.0]	14	4.9

Table 4.2 Mean relapse rates for patients taking azathioprine + 5-ASA and azathioprine alone

Group Analysis	N=	Mean relapse rate/ year follow up	Range (relapse rate)	SD	't' Test (AZA+5ASA vs. AZA alone)	Mean AZA dose (mg/kg)
UC						
(AZA + 5ASA)	55	0.21	0-1.3	0.31	P=0.69	1.75
UC (AZA alone)	27	0.19	0-0.9	0.28		1.85
CD						
(AZA+5ASA)	48	0.27	0-7.5	1.0	P=0.97	1.7
CD (AZA alone)	56	0.30	0-5	0.8		1.8

Table 4.3 Comparison of patients who discontinued AZA therapy before and after 6 months into treatment

Side Effects						
N=	M:F	CD:UC	On 5ASA ?	Mean AZA dose (mg/kg)	Mean duration of AZA Rx (years)	
Discontinued after 6 months	17	7:10	10:7	8	1.6	4.4
Discontinued before 6 months	17	9:8	12:5	7	1.4	0.2

4.4 Discussion

Relapse rates per year of follow up were not statistically different between the AZA + 5ASA groups and the AZA alone group (in UC or CD groups) and our cumulative remission curves compare well with other studies which have looked at remission maintenance with 5-ASA and AZA (Messori et al, 1994; Pearson et al. 1995; d'Albasio et al, 1997; Jean-Pierre Gendre et al, 1993; M.C.M.Rijk et al. 1992; Yoram Bouhnik et al, 1996). It would therefore appear that 5-ASA compounds afford no additional clinical benefit on relapse rates in patients suffering from either UC or CD. This study used data from a large database of IBD patients who were well matched for disease type and distribution, and there were comparable numbers of patients taking 5-ASA compounds to those who were not (table 1). However, this is a retrospective analysis of 2 groups of patients that may be selectively biased. It could be suggested that patients taking AZA alone were patients with more aggressive disease who were treated early on with AZA rather than using 5-ASA's. However, patients who were taking 5-ASA in addition to AZA did not have a more aggressive disease course from the standpoint of previous relapses and anatomical extent of disease. It was not possible to elucidate the impact of 5-ASA compounds on long-term malignancy risks in these patients because of the retrospective nature of the study. Although there are recent studies advocating the protective role of 5-ASA drugs in the development of colorectal malignancy(Davis et al. 1992; Ekbom, 1995; Moody et al. 1996; Andrianopoulos et al. 1989; Eaden et al. 2000), it still remains uncertain whether this is a direct effect of 5-ASA drugs or merely as a result of prolonged disease remission. Interestingly there have been reports of the potential role of folate supplementation in patients' taking sulphasalazine (a competitive inhibitor of folate

absorption) to reduce the incidence of dysplasia and cancer in chronic UC(BA Lashner et al. 1989). The role of 5-ASA's, especially sulphasalazine, in the prevention of colorectal malignancy is controversial and currently firm evidence is lacking. It is worthy to note that one case of dysplasia reported here was in a patient taking sulphasalazine, there was no obvious excess occurrence of malignancy in our group of patients taking sulphasalazine.

There has been growing interest over the last few years of the possible interaction between 5-ASA compounds and the enzyme TPMT. It has been reported that TPMT is potently inhibited by derivatives of benzoic acid (Woodson et al. 1983). Indeed there is convincing evidence that it is the 3-,4-, and 5-ASA components, that inhibit TPMT rather than other carrier molecules that are involved in the drug interaction (Szumlanski and Weinshilbourn 1995). In-vitro analysis has highlighted significant interactions with TPMT and balsalazide (Lowry et al. 1999), olsalazine(PW Lowry et al. 1999) and sulphasalazine (Lewis et al, 1997), and it is likely that mesalazine has a similar effect despite lack of data. In our study, the majority of patients were taking mesalazine, with smaller numbers taking sulphasalazine, balsalazide and olsalazine and it would appear that there is no evidence of any clinical risk from this potential drug interaction. Clearly, caution should be exercised whenever 5ASA drugs are being used in conjunction with AZA or 6-MP, especially if more potent inhibitors such as olsalazine and balsalazide are being used. The importance of TPMT inhibition by these drugs will be especially important in the minority of patients (up to 10%) who have genetically low levels of TPMT.

In summary, it is now important to follow up this study with a prospective double blinded and placebo controlled study to further validate these results.

Such a study will need to be multi-centre to answer adequately the question posed, and can be designed either to withdraw 5-ASA from patients maintained in remission on AZA+5-ASA in a randomised manner, or to commence AZA with or without 5-ASA at the initiation of immunosuppressive therapy.

Chapter 5. Azathioprine use in pregnancy in inflammatory bowel disease patients

5.1 Introduction

AZA is regarded as an important second line therapy in patients with steroid refractory or dependent IBD.

AZA has been reported to be safe during pregnancy in organ transplant patients and rheumatology patients, but there is clear paucity of similar data in IBD patients. Indeed, in animal models including rabbits, mice and rats AZA has been shown to be teratogenic, albeit at much higher doses mg/kg than is used in clinical practise in IBD patients. The reported occurrence of foetal abnormalities from patients taking AZA is somewhat varied. Prematurity for example has been reported in up to 22% of pregnancies while other complications such as cardiac, skeletal and haematological abnormalities occur between 9-17% of pregnancies (Alstead et al. 1990; O'Donnell et al. 1985; Ramsey-Goldman et al. 1993; Steven et al. 1979; Golby 1970).

Though most gastroenterologists consider the use of AZA to be relatively safe in pregnancy, variation in practice exists as to whether AZA therapy should be continued throughout pregnancy or omitted before conception. In this section we report on our experience of pregnancy outcome in patients who had conceived while on AZA therapy.

5.2 Materials and methods

A database of AZA using IBD patients (n=223; UC=104; CD=119) was searched and identified 11 patients suffering from IBD (8 CD, 3 UC) who had conceived while taking AZA. One patient had become pregnant twice, giving a total of 12 pregnancies. Antenatal and postnatal details as well as duration of drug therapy were obtained from the case records and from their respective general practitioners.

5.3 Results

There were 12 pregnancies from 11 women – mean age at conception was 29 years (range 26-33 years), eight had CD, and three UC. There were 10 live births from 12 pregnancies. One patient underwent a voluntary termination because of social reasons, and one patient underwent an emergency operation for an ectopic pregnancy. In neither case were the foetuses abnormal. At the time of pregnancy mean duration of AZA therapy was 3.5 years (range 0.6-6.4 years) and mean disease duration was 8 years (range 2-20 years). Only 2 patients had undergone surgery for their IBD before conception – both having a right hemicolectomy and ileal resection for ileocolonic CD (Table 5.1).

All but one was taking concurrent 5-ASA compounds – either mesalazine or sulphasalazine, while 3 other patients were, in addition, on low dose prednisolone ($\leq 10\text{mg od}$) (Table 5.1). In 8 of the 12 pregnancies, AZA was taken for the duration of gestation, mean AZA dose 1.9mg/kg (range $1.5\text{-}2.3\text{ mg/kg}$). Out of the remaining 4 pregnancies, one had emergency surgery for an ectopic pregnancy at 6 weeks gestation and had previously had abdominal surgery; another stopped AZA at 5 weeks gestation, because of AZA induced headaches. One patient stopped her AZA at 6 weeks after concerns over breast-feeding and the fourth patient underwent a voluntary termination for social reasons. There were no medical antenatal complications (Table 5.2).

Only one pregnancy did not go to term, delivered at 37 weeks. One patient delivered by caesarian section and this was performed electively at 39 weeks; the remaining

patients had normal vaginal deliveries. Mean birth weight was 3450 grams (range 3140-4100 grams), with 6 boys and 4 girls.

Excessive or serious infections in infancy, suggesting immunodeficiency, were not reported in any of the children. However, Postnatal complications were noted in 2 births – namely one baby with hypertrophic pyloric stenosis, requiring emergency surgery within the first week of life, and the other with an asymptomatic atrial septal defect which has since closed spontaneously (Table 5.3).

Table 5.1 Patient Details

Patient	Age at delivery (yr)	Diagnosis	Disease extent	Disease duration (yrs)	Previous surgery
1 SL	30	CD	Ileocolonic	3.3	+
2 MD	26	UC	Pan	3.4	-
3 LB	28	CD	Colitis	10.9	-
4 MG	33	CD	Ileal	18.6	-
5 AR	32(ectopic pregnancy)	CD	Ileocolonic	20.0	+
6 GC	27	UC	Left sided	1.4	-
7LC	26	CD	Ileocolonic	3.5	-
8 SC	36	UC	Left sided	16.0	-
9 JR*	33,34	CD	Ileal	8.3, 9.3	-
10 LG	27	CD	Colitis	5.5	-
11 LF	32	CD	Ileocolonic	3.0	-

* Patient had 2 pregnancies

Table 5.2 - Azathioprine and concurrent drug treatment

Patient	Duration Rx before pregnancy (years)	Duration during pregnancy (weeks)	Dose (mg/kg) AZA	Concurrent 5ASA?	Concurrent Prednisolone ?
1 SL	2.1	40/40	1.8	Mesalazine	-
2 MD	0.6	40/40	2.1	Mesalazine	-
3 LB	2.4	6/40	2.3	Mesalazine	10mg
4 MG	2.0	40/40	1.7	No	-
5 AR	3.1	6/40	2.0	Sulphasalazine	-
6 GC	1.0	40/40	1.5	Mesalazine	-
7LC	1.9	40/40	1.7	Mesalazine	7.5mg
8 SC	3.6	5/40	1.6	Sulphasalazine	9mg
9 JR	5.4	40/40	1.5	Mesalazine	-
	6.4	41/40	1.5	Mesalazine	-
10 LG	1.0	8/40	1.6	Sulphasalazine	-
11 LF	2.4	40/40	2.3	Mesalazine	-

Table 5.3 - Pregnancy and Postnatal details

Patient	UC/ CD	Pregnancy No.	Disease Duration (Yrs)	Antenatal Complications	AZA stopped?	Duration of Rx (Yrs)	Previous Surgery	Gestation (wk)	Delivery	Postnatal complications
1	CD	1	3.3	None	N	2.1	+	40/40	NVD	Nil
2	UC	2	3.4	None	N	0.6	-	40/40	NVD	Nil
3	CD	3	10.9	None	At 6/40	2.4	-	40/40	NVD	Nil
4	CD	4	18.6	None	N	2.0	-	40/40	NVD	Nil
5	CD	5	20.0	Ectopic pregnancy	N	3.1	+	6/40	Emergency Op	N/a
6	UC	6	1.4	None	N	1.0	-	40/40	NVD	Nil
7	CD	7	3.5	None	N	1.9	-	40/40	NVD	Nil
8	UC	8	16.0	Headaches 2° to AZA	At 5/40	3.6	-	4/40	NVD	Nil
9	CD	9 10	8.3 9.3	None None	N N	5.4 6.4	-	40/40 41/40	NVD NVD	Nil Pyloric stenosis
10	CD	11	5.5	Voluntary termination	N	1.0	-	8/40	Termination	N/a
11	CD	12	3.0	None	N	2.4	-	40/40	NVD	ASD

5.4 Discussion

AZA is a synthetic antimetabolic drug and its effects have been well documented in pregnant animals, namely, mice rabbits and rats. At high doses of around 20mg/kg, it impairs tissue, organ and placental differentiation in rats, although congenital abnormalities have not been reported(Cote et al. 1974; Gross et al. 1977). In rabbits and mice, teratogenicity has been shown (predominantly skeletal abnormalities, haemopoietic and thymic depression) even if AZA was given up to the midpoint of gestation(Tuchmann-Dupleiss and Mercier-Parot 1966).

There is no doubt that AZA crosses the placenta and expressed in breast milk in mothers taking AZA. However, the concentrations in placental tissue are small representing up to 14% of the orally administered dose, and thus it appears unlikely that such metabolite concentrations will have a clinically significant effect on the foetus (Rosenkrantz et al. 1967).

In humans, AZA's effect on pregnancy and offspring has been predominantly looked at in organ transplant patients - usually taking AZA and prednisolone. Here, cytogenetic analysis has detected chromosomal aberrations in offspring, but long term follow up has not revealed further problems in these infants (Willoughby et al. 1971). There is no current evidence of fertility problems in patients taking AZA (Thiersch 1962). Immunological abnormalities have also been reported in the newborn's of these patients(Golby, 1970; Williamson and Karp, 1981), constituting lymphopenia, thymic hypoplasia and bone marrow hypoplasia, but all of these have subsequently resolved later in infancy. Unfortunately, there is a lack of reports of *detailed* immunological assessment in these patients.

There have also been isolated anecdotal reports of skeletal abnormalities such as type 1 preaxial polydactyly in children, whose mothers have been taking AZA (Schwartz 1966). However such abnormalities often have a polygenic aetiology and it is difficult to attribute such isolated abnormalities directly to AZA use - especially when equivalent doses used in humans (2-4 mg/kg) do not appear to show teratogenic effects in animal models. Indeed the differences in clinical effects between animal models and humans may well be attributed to different enzymic handling of AZA and 6MP.

In this study, there were no premature births recorded, although there were 2 infants that suffered congenital abnormalities. However there were no skeletal deformities recorded in any of the infants, and it is plausible that these infants had sporadic abnormalities, rather than these being directly as a result of AZA. The lack of power of this study combined with the occurrence of 2 congenital abnormalities suggests we should be a little less certain about the absolute safety of AZA in pregnancy in IBD. Previous studies in groups of IBD patients taking AZA have also recorded healthy offspring, although there have been cases of viral infections - Hepatitis B and Cytomegalovirus in the mother and subsequently the offspring (Saarikoski and Seppala, 1972; Willoughby et al. 1971). In summary, AZA therapy appears to be associated with successful outcome of pregnancy in the majority of patients, though much larger numbers of reports need to be collated to have robust data on its use in pregnancy. Our own data relates to use of the drug in therapeutic doses of around 2mg/kg, but it must be remembered that higher doses are often used in the US. Our current practice with IBD patients who conceive while taking AZA, is to continue

therapy, on the basis that good control of the patients IBD is much more important for the well being of the foetus and pregnancy. It should be remembered that patients with CD have higher rates of spontaneous abortion and stillbirths, and that controlling disease activity may reduce these risks (Alstead et al. 1990). AZA is generally not started *during* pregnancy, as there are potential risks from AZA related adverse drug reactions, which occur in up to 10% of patients starting therapy. All patients get an AZA information sheet from our pharmacy, explaining risks and benefits of AZA therapy, and patients are reassured that with increasing numbers of reports, it appears AZA is compatible with a normal pregnancy and healthy offspring

Chapter 6. Effects of Azathioprine on progeny of male IBD patients

6.1 Introduction

Clinicians are not under any form of compulsion to report outcome of administration of drugs during pregnancy to a central monitoring authority. Therefore, information regarding pregnancy outcome is reliant upon voluntary reporting – and therefore subject to an obvious bias and tendency to report unfavourable outcomes, rather than routine findings after such treatment. Hence there is a relatively small amount of information regarding pregnancy outcomes in patients taking AZA. The majority of reports have been in transplant patients where the origins of AZA usage began. Furthermore, even less attention has focussed upon the progeny of fathers who have received similar AZA therapy. Again, the majority of current information is available on renal transplant patients, but numbers remain small. There is only one study to date that has examined progeny of male IBD patients taking 6-MP. This has urged caution in fathers taking 6-MP within 3 months of conception due to an increase in pregnancy related complications (Rajapakse et al 2000).

Studies in rats have also suggested that spermatogenesis is effected by AZA and 6-MP (Karl et al 1991) , calling for further evaluation of fertility in male patients receiving AZA or 6-MP. Although male fertility has been reported to be reduced in the rheumatological literature (Janssen et al 2000), more recent work studying male fertility in IBD patients on AZA has been reported to be normal (Dejaco et al 2001).

The aim of this study was to examine the clinical outcomes of progeny of fathers who received AZA therapy for their IBD.

6.2 Patients and Methods

Male IBD patients taking AZA or 6-MP were identified from an IBD database.

Patients were excluded if they were aged below the age of 18 years or above the age of 65 years. A total of 81 male patients (47 UC, 34 CD, median age 37 years, range 21-65 years) were surveyed.

Patients were contacted by post and asked to fill in (anonymously) a questionnaire regarding AZA usage, partners' conception, and offspring health. Other information obtained included concomitant medications, age at partners conception, disease distribution duration and duration of AZA therapy.

6.3 Results

A total of 81 letters were sent to 81 male IBD patients. Forty-nine (60%) patients replied. There were a total of 13 documented pregnancies from 10 fathers (6CD:4UC, median age at partners conception 33 years, range 24-55 years) and 10 mothers (median age at conception 28 years, range 22-40 years). All male patients were taking AZA at the time of their partners' conception. None of the patients' partners were taking immunosuppressive agents.

Median dose of AZA was 1.9mg/kg (range 1.2-2.3 mg/kg); median duration of AZA therapy at partners' conception was 3.5 years (range 1.0-6.1 years). Seven out of the 10 fathers were taking a 5-ASA drug (mesalazine, median dose 800mg tds, range 400-800 mg tds). None were taking sulphasalazine. There were only 2 patients taking low-dose steroid therapy (prednisolone 10mg/day). Two patients were taking AZA alone. Three of the fathers had 2 progeny while they were taking AZA. There were no premature births or birth defects in this cohort. One father's progeny had autism, but his partner suffered from epilepsy was taking carbamazepine during the pregnancy and was also suffering from hypothyroidism (with thyroxine replacement). Another father's progeny developed a cerebral tumour (astrocytoma) at the age of 10 years old. All of the other progeny were clinically well, with no reported problems with fertility and conception. Except the one mother who suffered from epilepsy, all the other mothers were well and not taking any medications during the pregnancy. Table 6.1 summarises these result.

Table 6.1 – Patient characteristics

Patient No.	Age @ conception (years)	AZA dose (mg/kg)	Duration of AZA @ conception	Concurrent 5-ASA? (mg, tds)	Concurrent steroids (mg)	UC/CD (distribution)	Offspring	Childhood illness?	Maternal illness during pregnancy	Mother's medications?
1	24	1.4	1.9	No	Pred 10	CD (colonic)	1	Nil	Nil	Nil
2	41	2.0	4.1	Mesalazine	No	UC(proctitis)	2	1 st child – autism	Hypothyroidism + Epilepsy	Thyroxine & Carbamazepine
3	31	1.5	1.6	Mesalazine	No	UC (pan-colitis)	1	Nil	Nil	Nil
4	31	2.1	6.0	Mesalazine	No	CD (colonic)	2	Nil	Nil	Nil
5	55	2.1	2.9	Mesalazine	No	UC (proctitis)	1	Nil	Nil	Nil
6	32	1.4	3.8	Mesalazine	Pred 7.5	CD (ileocolonic)	1	Nil	Nil	Nil
7	41	2.3	6.1	No	No	CD (colonic)	1	Nil	Nil	Nil
8	45	1.2	2.8	Mesalazine	No	CD (colonic)	1	Nil	Nil	Nil
9	28	1.8	1.0	Mesalazine	No	UC (pan-colitis)	2	Nil	Nil	Nil
10	34	2.1	3.5	No	No	CD (proctitis)	1	Astrocytoma	Nil	Nil

6.4 Discussion

There were a total of 13 pregnancies and thirteen live births, with 10 fathers currently taking AZA at the time of their partner's conception. All the fathers were taking AZA for a minimum of 1 year.

There have been very few studies that have looked at teratogenesis and clastogenesis resulting from paternal use of AZA. There have been several small papers describing experiences pregnancies in the partners of male renal transplant patients – more than 90% of births were overtly normal, with spontaneous abortion, neural tube defects and trisomy 21 making up the remainder of birth complications (Eslami et al. 1976; Papoff et al. 1977; Whetham et al. 1983). To date there has been only one study that has described outcome of pregnancy when fathers are treated with 6MP for IBD (Rajapakse et al 2000). They describe a single centre retrospective experience over a period of 27 years. They compared the outcome of pregnancies fathered by IBD men taking 6MP (subdivided into group 1a (n=13), fathers who conceived within 3 months of 6MP use and group 1b (n=37), those who conceived at least 3 months after 6MP was stopped) versus IBD men not taking 6MP. The only complications they reported were in group 1a. The 4 complications included 2 spontaneous abortions and 2 congenital abnormalities (acrania and a missing thumb). This study was criticised because of its retrospective nature and the small numbers of patients in the 6MP group.

In this cohort, there were only 2 progeny who later developed complications – 1 with autism, and the other progeny who developed a cerebral astrocytoma at the age of 10

years. While it could be argued that these may have been associated with the fathers AZA therapy, these 2 conditions are relatively common childhood conditions and probably would have occurred even if the father was not taking AZA. The aetiology of autism is unknown, although there are reports of associations with maternal hypothyroidism (Gillberg et al. 1992), and increasing evidence that environmental factors upon the mother during pregnancy may increase the risk of their children developing autism (London and Etzel 2000). There has also been speculation that there may be a predominance of autism in children who have ‘new variant inflammatory bowel disease’ (Wakefield AJ et al 1998). This latter report remains controversial with a more recent epidemiological study reporting conflicting evidence (Taylor B et al 1999). Malignant cerebral tumours are the second most common childhood cancers (after leukaemia’s) and their incidence in the UK is rising, accounting for around 300 cases per year (McKinney et al. 1998). Astrocytoma’s account for nearly 30% of newly diagnosed cases, making them the most common form of childhood cerebral tumour (Stiller et al 2000).

Detailed evaluations of fertility issues were not possible in this study, but there were no reports of infertility or problems with partners conceiving. Animal models have suggested that spermatogenesis is impaired – but this has been when higher doses of 3-5mg/kg AZA are used (Karl et al. 1991). Studies of spermatogenesis in humans have had conflicting results. A study of 34 renal transplant patients had spermograms which were not significantly different from healthy controls (Evans et al. 1975). Other smaller studies have reported significantly lower sperm quality in renal transplant recipients (Salant et al. 1976). Finally, studies in patients with rheumatoid

arthritis (Currey et al. 1974) and systemic lupus erythematosus (Masramon et al. 1980) revealed normal sperm counts in all of their cohorts.

This is a small cohort of male IBD patients who were taking AZA, and therefore it would be difficult to make substantial conclusions from this experience alone. Indeed, like the study by Rajapakse et al (Rajapakse et al 2000), this study can also be criticised because of its retrospective nature. This further highlights the difficulty in obtaining conclusive data from this type of patient group from a single centre. As stated earlier with pregnancy in IBD patients, a central database collecting information from multiple centres would help to give more conclusive information. However, these patients were well established upon their AZA therapy for several years and in addition were taking substantial doses of the drug (median dose 1.8mg/kg).

In summary, there does not appear to be any obvious serious implications for the progeny of IBD patients taking AZA. Before male patients are started upon AZA risks should always be explained to the patient, as well as the potential risks to any progeny they may sire – albeit seemingly low. More data is required and caution similar to maternal use of AZA should be exercised and individualised decisions taken. Though most men and women with UC and CD can expect a healthy child (Ludvigsson JF et al 2002), US studies have urged caution in fathers using 6-MP within 3 months of conception due to increase in pregnancy related complications (Rajapakse et al 2000, Korelitz et al 2001).

Chapter 7. Cyclosporin rescue therapy used in combination with azathioprine and corticosteroids in severe ulcerative colitis

7.1 Introduction

CyA is a fungal metabolite and is a powerful immunosuppressant. Its action is lymphocyte specific, inhibiting interleukin-2 and therefore the function of T helper cells. Thereafter, recruitment of cytotoxic T cell populations are inhibited as well as the release of other lymphokines (Baker and Jewell 1989).

Patients with a severe attack of UC, characterised by severe rectal bleeding, diarrhoea and fever (Edwards and Truelove 1963) will inevitably require hospitalisation and the administration of intravenous steroid therapy. Response to this intravenous treatment is in excess of 60% (Jannerot et al. 1985), leaving the remainder of patients with the options of curative surgery (i.e. panproctocolectomy) or the administration of intravenous rescue therapy with CyA. Intravenous CyA is far from widely accepted by the medical community, and to date there have been only two randomised controlled trials into the use of CyA in severe relapses of UC (D'Haens et al. 2001; Lichtiger et al 1994). Lichtiger et al (Lichtiger et al 1994) studied a total of 20 patients who were randomised to receive IV CyA (4mg/kg/day) or placebo, in addition to continued glucocorticoids for up to 14 days. Nine out of eleven patients receiving CyA improved after 7 days compared with 0 of 9 for the placebo arm. They

concluded that CyA was an effective treatment in the acute setting, but did not assess long-term follow-up on these patients. None of these patients receive AZA. D'Haens et al studied monotherapy with intravenous CyA versus intravenous glucocorticoids in severe attacks of UC. A total of 30 patients were studied (15 patients received CyA and 15 received glucocorticoid). Eight of the 15 patients who received intravenous methylprednisolone (40mg/day) responded after 8 days versus 9 of 14 who received CyA (4mg/kg/day). At 1 year, 7 of these CyA patients maintained their remission versus only 3 of 8 treated with methylprednisolone. It is of note that all the patients who received CyA continued on oral AZA, while only 37% of the methylprednisolone treated patients subsequently went on to receive AZA. Actis et al (Actis et al 2001) examined the safety and efficacy of azathioprine in the maintenance of cyclosporin-induced remission of UC as compared to remission attained by intravenous steroids. They found that discontinuation of AZA was similar in both groups advocating the safety of overlapping AZA, CyA and a reducing dose of oral steroid therapy. Their CyA group was particularly heterogeneous - some patients receiving AZA after their first relapse following CyA therapy and others receiving AZA at the time of initial CyA therapy, making the role of AZA in these patients unclear.

There are several uncontrolled trials that have shown promising initial response rates of 82% at day 7 and 69% response by 6 months (Kornbluth et al. 1994; Lichtiger et al. 1994). However there is not enough data to firmly conclude that CyA therapy is definitely beneficial in patients with severe UC.

Unfortunately there been contradictory studies, reporting much higher relapse rates recorded at 12 months and beyond (Baert and Hanauer 1994). In addition to this,

there are concerns over the number of potential toxicity from CyA therapy (Carbonnel et al. 1996). Successful treatment with CyA avoids the immediate necessity of a surgical procedure, but the relapse rate and colectomy rate subsequently may be high. To reduce this high relapse rate, the addition of oral AZA after remission has been achieved with CyA has been tried.(Cohen et al. 1999; Fernandez-Banares et al. 1996), but there are serious concerns about side effects including opportunistic infection such as pneumocystis carinii pneumonia.

The paucity of data currently available on patients receiving rescue CyA therapy in combination with AZA and corticosteroids compounds our indecisive use of this treatment regimen within the acute setting. The aim of this study was to report our experience of the use of intravenous CyA in acute UC in combination with AZA and steroids and to contribute further information as to its optimal use.

7.2 Patients and Methods

The hypothesis was that there is no difference in long term outcome in severe UC patients who received intravenous CyA who subsequently received AZA therapy compared to those patients who did not.

Between January 1994 and January 2001, 17 patients had received intravenous CyA for acute attacks of UC at the Western General Hospital, Edinburgh. The aim of this was to gather information regarding CyA use in UC patients. The decision to administer CyA was taken after detailed discussion with the patient about potential risks and benefits. In addition it was only patients expressing the strong desire to avoid surgery that were counselled in this fashion. Follow-up treatment with AZA and the potential alternative of proctocolectomy were also discussed. These discussions always involved a consultant caring for the patient (SG).

Information obtained was age, disease duration and anatomy, concurrent use of AZA, time to surgery, side effects from CyA, use of *Pneumocystis Carnii* (PCP) prophylaxis, CyA dosage and duration and previously published predictors of outcome(Travis et al. 1996) (CRP at 3 days, stool frequency at 3 days). This left 17 patients who had received intravenous CyA for acute UC. One patient had moved area and their case notes could not be located, leaving 16 patients who were evaluable (7 female, 9 male, mean age 33 years, mean disease duration 6.6 years, mean follow up 3.1 years).

All patients were refractory to treatment with a standardized intravenous steroid regimen of 60mg methylprednisolone given over 24 hours as a continuous infusion

(for a minimum of 5 days). The majority of patients receiving oral CyA received Neoral® micro emulsion preparation tablets, only 2 patients received oil based preparations. Only patients who responded initially to intravenous CyA were commenced on AZA, and all patients were given 5-ASA therapy (mesalazine 800mg tds). Our discharge practice for these patients involved oral triple immunosuppressive therapy (oral CyA, azathioprine, oral steroids). Patients were started on oral prednisolone at a dose of 40mg tapering by 5 mg every week (unless the patient relapsed and steroid doses were kept at a stable enough dose to achieve remission). At the same time oral CyA was commenced (and dose adjusted to serum levels) and maintained for a minimum of 4 weeks (median 6 weeks, range 4-15 weeks). AZA therapy was titrated to as close to 2mg/kg as was allowed by blood count and liver function test monitoring or patient side effects. Oral steroids were tapered first followed by CyA. AZA therapy was continued thereafter with a 5-ASA drug. Throughout the duration of CyA therapy, PCP prophylaxis was given in the form of Septrin 960mg on alternate days.

7.2.1 Statistical analysis - Statistical analysis was by Mann-Whitney test (for non parametric data) and time to first relapse data was analysed by Kaplan-Meier survival curves and log rank testing. Statistical significance was taken for $p < 0.05$. All statistical analyses were performed using Minitab release 13.20 software.

7.3 Results

This study includes 16 patients (7 female:9 male, median age 35 years, range 22-43 years) who were treated for acute severe UC with intravenous CyA. All met Truelove and Witts criteria for severe acute UC. The median disease duration was 5.4 years (range 0.9-25 years). All patients were refractory to IV methylprednisolone 60mg/24hr and subsequently treated with CyA at a dose of 4mg/kg. Trough CyA levels were checked 72 hours into IV CyA, and weekly for oral CyA patients. Our laboratory reference range is 120-300 nmol/l. Nine patients had this subsequently reduced according to CyA levels (median CyA dose 267mg, range 200-400mg). The median duration of intravenous CyA therapy was 7 days (range 3-16 days), with subsequent oral CyA (median dose 350mg, range 150-400mg) being continued in 9 patients (median duration 6weeks, range 0.3-15 weeks). Median follow-up duration after initial CyA therapy was 2.6 years (range 0.5-7 years). Two of the 9 patients received oil-based CyA preparations, while the remaining 7 patients received microemulsion preparation (Neoral®). These 9 patients were simultaneously started on oral AZA (median dose 1.8 mg/kg, range 1.2 – 2.1 mg/kg) in conjunction with oral CyA oral steroids and mesalazine. Although an AZA dose of 2mg/kg body weight was the primary goal, dose adjustment was necessary in some patients due to side effects (e.g. nausea, slight abnormalities in LFT's, and neutropenia). The remaining 7 patients underwent surgery (6 had panproctocolectomy and ileostomy, while one patient had a panproctocolectomy and ileo-anal pouch formation) before oral CyA and oral AZA therapy was instituted. Five patients were admitted with their first attack requiring CyA rescue therapy. All patients had a flare-up of their known UC - 14 patients had pan colitis, and 2 had left sided colitis. The median lead-in-time for

patients (duration of symptoms before admission) was 18 days (range 7-60 days).
These characteristics are summarized in table 7.1.

Table 7.1 – Patient characteristics

Variable	Median value (range)
Male: Female	9:7
Age	35 years (22-43)
Disease Duration	5.4 years (0.9-25)
Number admitted with 1 st attack	5
CRP @ 3 days	4.4 (1.5-18)
Stool frequency @ 3 days	8 (3-9)
Disease distribution (L-left side, P-pan colitis, D- distal)	L – 1 P – 14 D – 1
Duration of IV CyA	7 days (3-16)
Duration of oral CyA	6 weeks (4-15)
Duration of hospital stay	27 days (9-34)
Number taking PCP prophylaxis	16
Lead-in-time	18 days (7-60)
Patients requiring surgery	7 (44%)

Figure 7.1 shows a Kaplan Meier survival plot for time to first relapse for all patients and patients who had received a combination of CyA and AZA. All patients in long-term remission were taking AZA. Fifty-six percent of patients retained their colon.

Comparisons were made between patients who presented with their 1st attack with patients who had more than one attack of severe colitis (median number of attacks = 3, range 2-5) – this data is shown in figure 7.2. Log rank analysis was again not statistically significant ($\chi^2=0.06$, $p=NS$).

Comparisons were also made between patients who had ≤ 7 days of intravenous steroid ($n=10$, median duration 6 days; range 5-7 days) versus patients having >7 days intravenous steroids ($n=7$, median duration 12 days; range 8-16 days) prior to commencement of CyA therapy. Figure 7.3. Illustrates the survival curves for this data. Log rank analysis was not statistically significant ($\chi^2=0.0005$, $p=NS$).

Comparative Kaplan-Meier survival curve analysis for time to first relapse (not shown) were performed with other subgroups, including stool frequency at 3 days (≤ 8 stools per day versus >8 stools per day) and CRP at 3 days (CRP ≤ 4.5 versus CRP >4.5) – neither reached statistical significance ($\chi^2=0.3$, $p=NS$ and $\chi^2=0.2$, $p=NS$ respectively).

Finally, comparisons were made between patients who underwent surgery and patients who had successful rescue therapy with AZA. Median age, lead-in-time, and median CyA levels were not statistically significant between the two groups. Of interest, was that predictive markers of the need for surgery (CRP and bowel

Figure 7.1 - Survival curve for time to 1st relapse for all patients, patients taking AZA+CyA combination and CyA alone

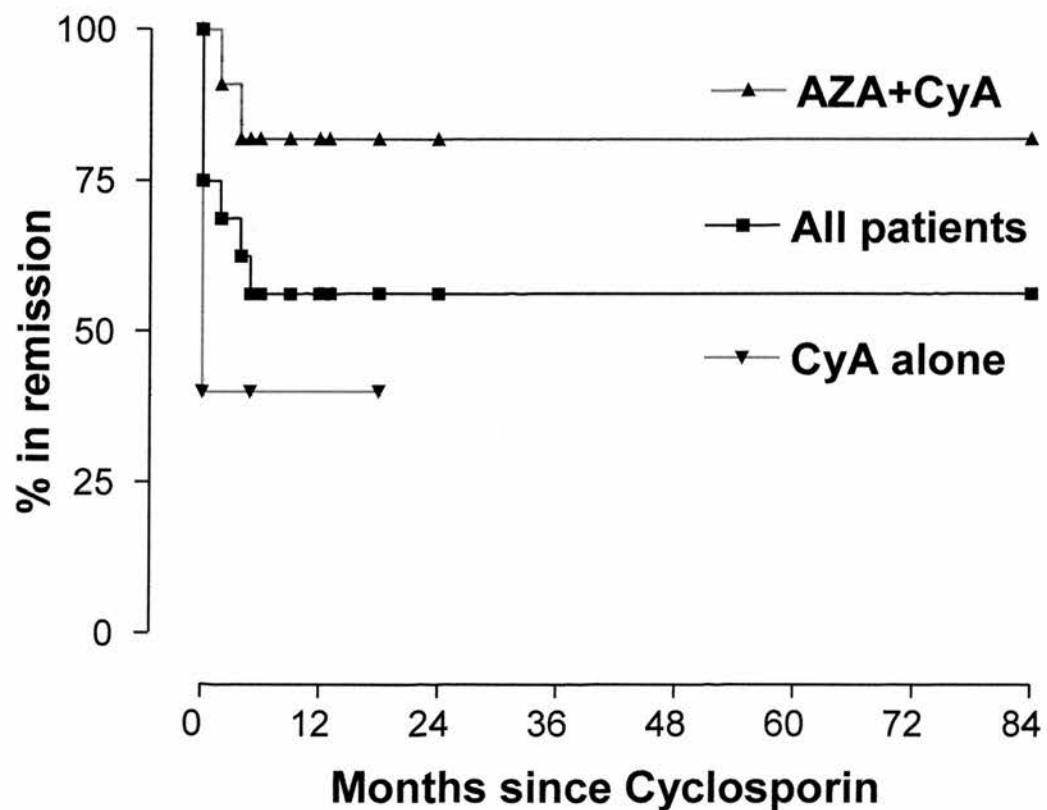


Figure 7.2 - Survival curve comparison between patients presenting as 1st attack(n=6) versus patients presenting after previous relapses (n=10).

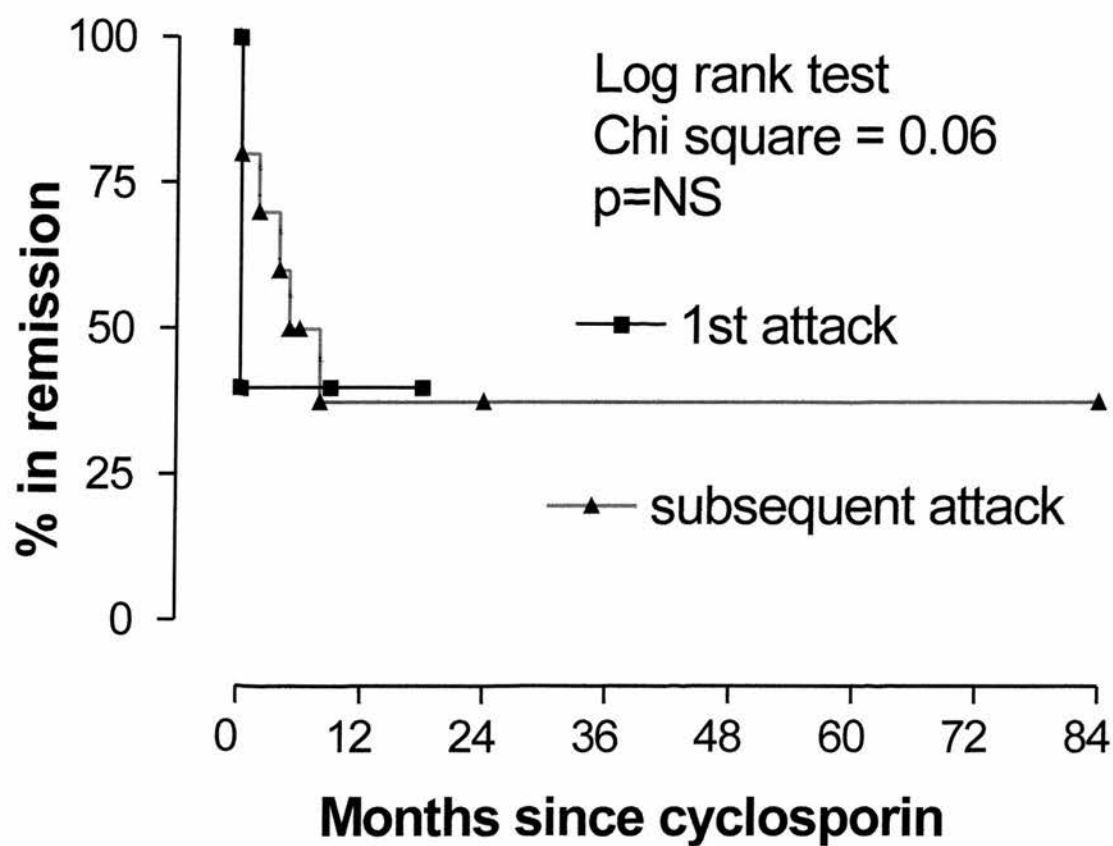
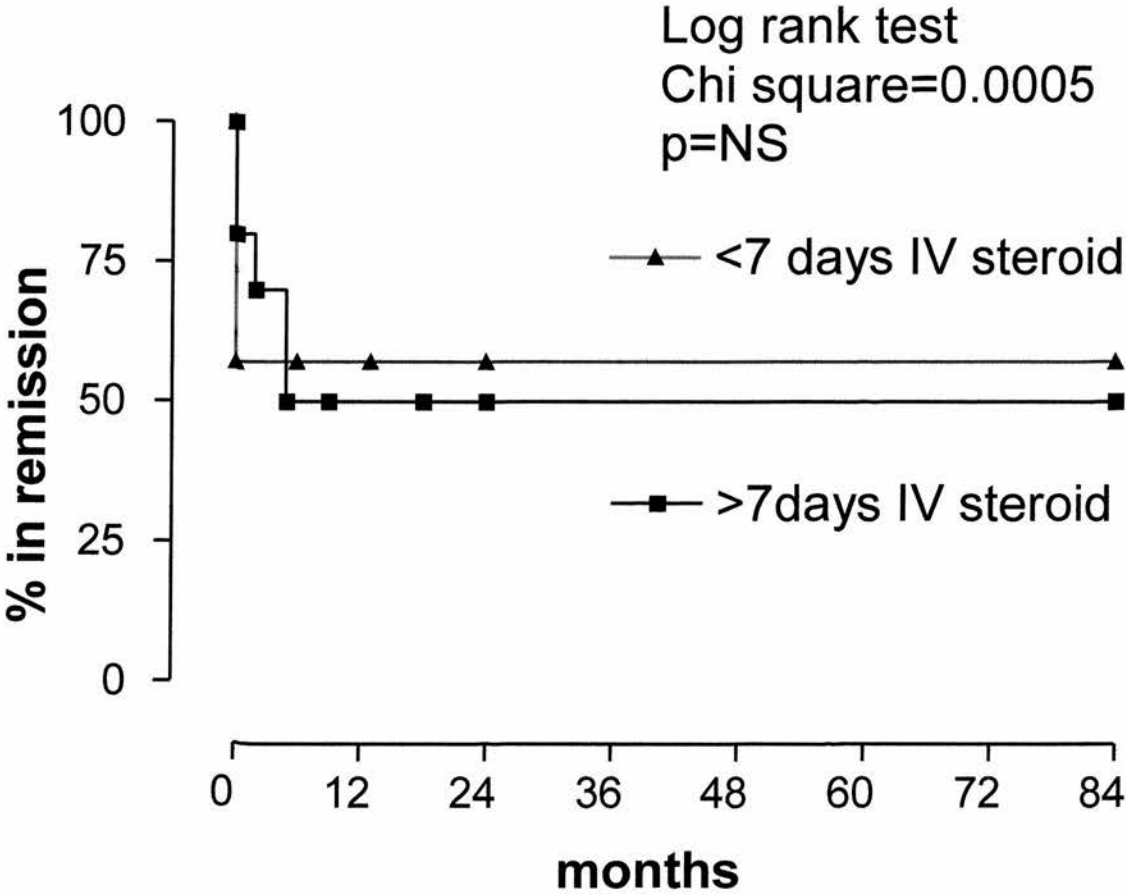


Figure 7.3 - Survival curve comparison (time to first relapse) for duration of initial IV steroid use (<7 days, n=7 versus >7 days, n=9)



frequency at 3 days). Bowel frequency at 3 days just failed to reach statistical significance, with a median CRP of 5.2 in the surgical group (MWT, $p=0.06$). The median CRP was higher in the surgical group, although this did not reach statistical significance (MWT, $p=0.7$). Median duration of intravenous CyA was higher in the non-surgical patient group when compared to the surgical group (7 days versus 4 days, MWT; $p=0.05$). These results are summarized in Table 7.2.

**Table 7.2 - Comparison of surgical patients and non-surgical
(rescue successful) patient**

	Surgical Median (range)	Non-surgical Median (range)	MWT (p=)
N=	7	9	
Median Age (years)	36 (22-43)	28 (22-41)	0.07
Lead-in-time (days)	18 (7-60)	18 (14-35)	0.9
CRP @ 3 days	5.2 (3-19)	2.6 (1.5-16.0)	0.7
Bowel frequency @ 3 days	10 (3-12)	6 (4-8)	0.06
Duration of IV CyA	4 (3-10)	8 (5-16)	0.05*
Patients taking AZA	2	9	
Median dose AZA (mg/kg)	1.6 (1.2-2.1)	1.8 (1.6-2.1)	0.8
CyA level	250	290	0.5

7.4 Discussion

CyA rescue therapy has previously been shown to be extremely useful in inducing remission and saving patients with UC from surgery; studies have reported early response rates in between 60 to 80% (Lichtiger and Present 1990; van Gossum et al. 1996; Sandborn 1995). Our series also confirms this relatively high initial response rate (11 patients, 69%). Interestingly our long-term success rate was more impressive than results from Baert et al (Baert and Hanauer 1994) and comparable to that found by other studies (Sandborn 1995) (9 patients avoided surgery – 56% - with a follow up of 3 years).

Side effects are a significant cause of morbidity in patients treated with CyA (Cohen et al. 1999) and our experience was similar to that published previously. Figure 7.4 illustrates CyA related side effects in our patient group. Hypomagnasaemia, abdominal pain, tremor and myalgia being the most common, but more importantly none of these side effects were deemed severe enough by the patient or physician to merit discontinuation, something that may have been aided by strict and regular CyA level monitoring and dose adjustment in this group. One patient developed mild epigastric discomfort associated with a raised amylase. It was unclear whether this particular side effect was due to CyA or concurrent AZA. This resolved spontaneously and did not require dose adjustment of either immunosuppressants. In addition, this study involves a relatively small number of patients and therefore may be subject to type II errors. The small number of patients per year in this study as compared to other studies (Cohen et al 1999, Actis 2000) does not reflect a strict pre-selection criteria but rather a much lower trend in the UK of cyclosporin use in

general. It should be noted that other groups have reported serious side effects with similar immunosuppressive drug regimens and in one Belgium study, 3 deaths were attributed to opportunistic infection (1 PCP, 2 aspergillus infection) and 1 anaphylactic reaction that the patient survived. This study cohort contained 86 patients and it is of note that the patients who developed infectious complications were not given antibiotic prophylaxis (Arts et al 2001).

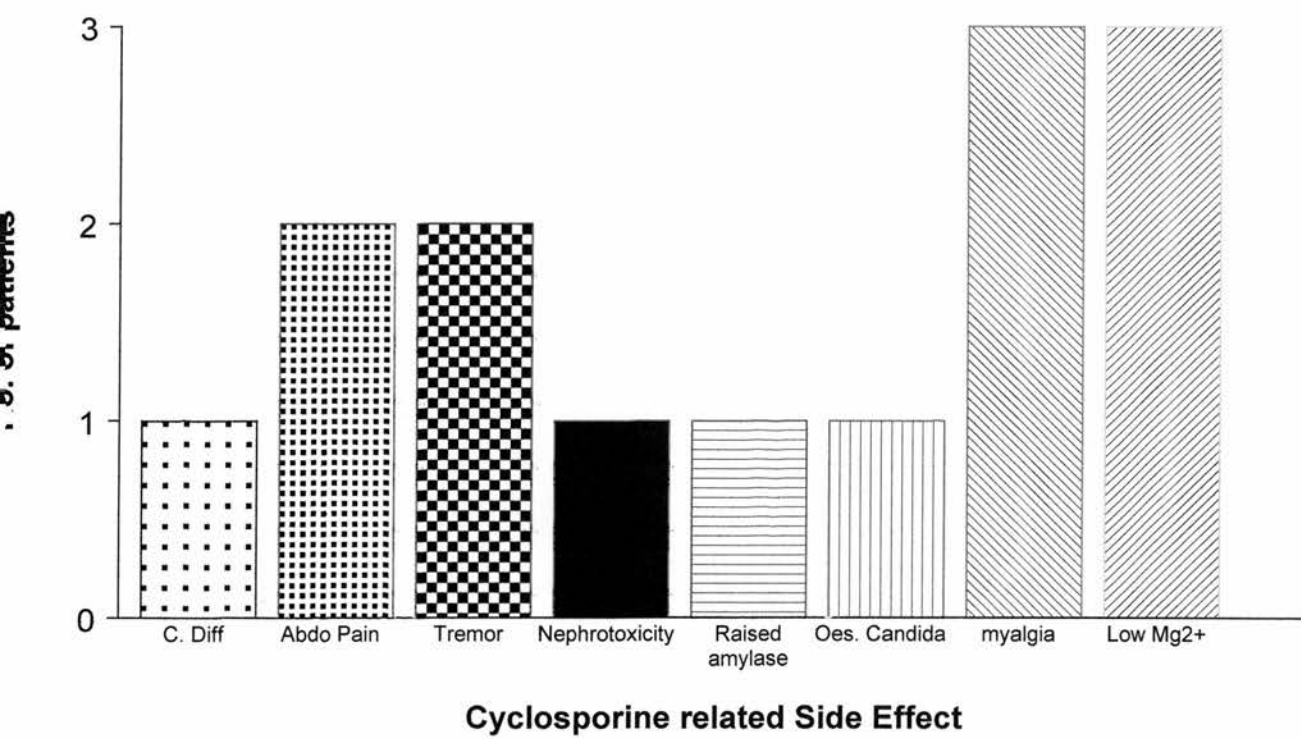
The use of AZA alone has been shown to be effective in inducing and maintaining remission in UC patient's outwith of CyA use (Sandborn, 1998). In this study only 2 (22%) patients who were started on concurrent AZA later required surgery, with the remaining 7 patients maintaining clinical remission to date (and also exhibited a significant steroid sparing effect). In the opinion of the author, this further advocates the concurrent use of oral AZA in the setting of remission induction by IV CyA. This is in light of the high percentage of patients successfully rescued in this series that remained in remission long-term and is much higher than the colectomy rate previously published with CyA therapy without subsequent oral AZA (Gurudu et al. 1999). There was only one patient who had to discontinue AZA because of side effects (nausea) and that patient underwent surgery.

From our survival curves it seems that if patients go into remission by 4 weeks into therapy with CyA and AZA then the majority (56%) will maintain this remission long term. In addition, this data would support the potential role of oral CyA as a good bridging therapy to AZA, a role that steroid monotherapy has previously been used for. Figure 7.5 summarizes the outcome of these patients. Table 7.3 summarizes the follow-up time for the 9 patients who avoided surgery.

Of note also are the predictive markers of requirement of surgery. Median bowel frequency at day 3 was significantly higher in patients who finally underwent surgery. Median CRP at day 3 was also higher in the surgery group (but did not reach statistical significance), confirming results by Travis et al (Travis et al. 1996) that these are valuable markers in predicting patients that will finally need to undergo surgical intervention.

Oral CyA is rarely used as a second-line agent (like AZA) in treating steroid refractory or resistance disease. This has been compounded by mixed reports concerning the bioavailability of different oral CyA preparations in patients as well as different absorption rates in different ethnic groups (Choc, 1997; Curtis et al. 1999; Palma-Aguirre et al. 1997). In particular this led scepticism upon absorptive rates of CyA in IBD patients and hence its relative effectiveness when used in such patients. Latteri et al revealed that the pharmacokinetic profiles of CyA microemulsion is broadly similar in IBD and healthy volunteers (Latteri et al. 2001). The clinical efficacy of oral CyA in this series confirms this, with the majority of patients (71%) remaining in remission long-term and would support the potential role of oral CyA maintenance therapy as an AZA substitute in patients who can not tolerate AZA.

Figure 7.4 - Summary of Cyclosporin related side effects



In summary, the use of CyA in acute severe UC is now a well-established alternative to early surgery – the current standard practice (Kornbluth et al. 1994). It should be remembered that patients quality of life has been shown to be higher in patients kept in remission with CyA when compared to those patients who have undergone surgery (Cohen et al. 1999). While the significant side-effect profile of CyA should be always be borne in mind, this study further strengthens both the role of CyA as a 3rd line drug therapy option that significantly reduces the need for surgery, while having a relatively good safety profile when monitored carefully and of triple immunosuppressive therapy with AZA in maintaining the remission achieved by IV CyA. If oral CyA is used, this should be followed by AZA therapy to avoid the high relapse rate, and such vigorous immunosuppression, while associated with potential risks, can result in satisfactory long-term outcome. In addition, oral CyA should be considered as an alternative to oral AZA in those patients who are unable to tolerate it.

Figure 7.5 - Flow-chart summarizing outcomes of UC patients treated with CyA

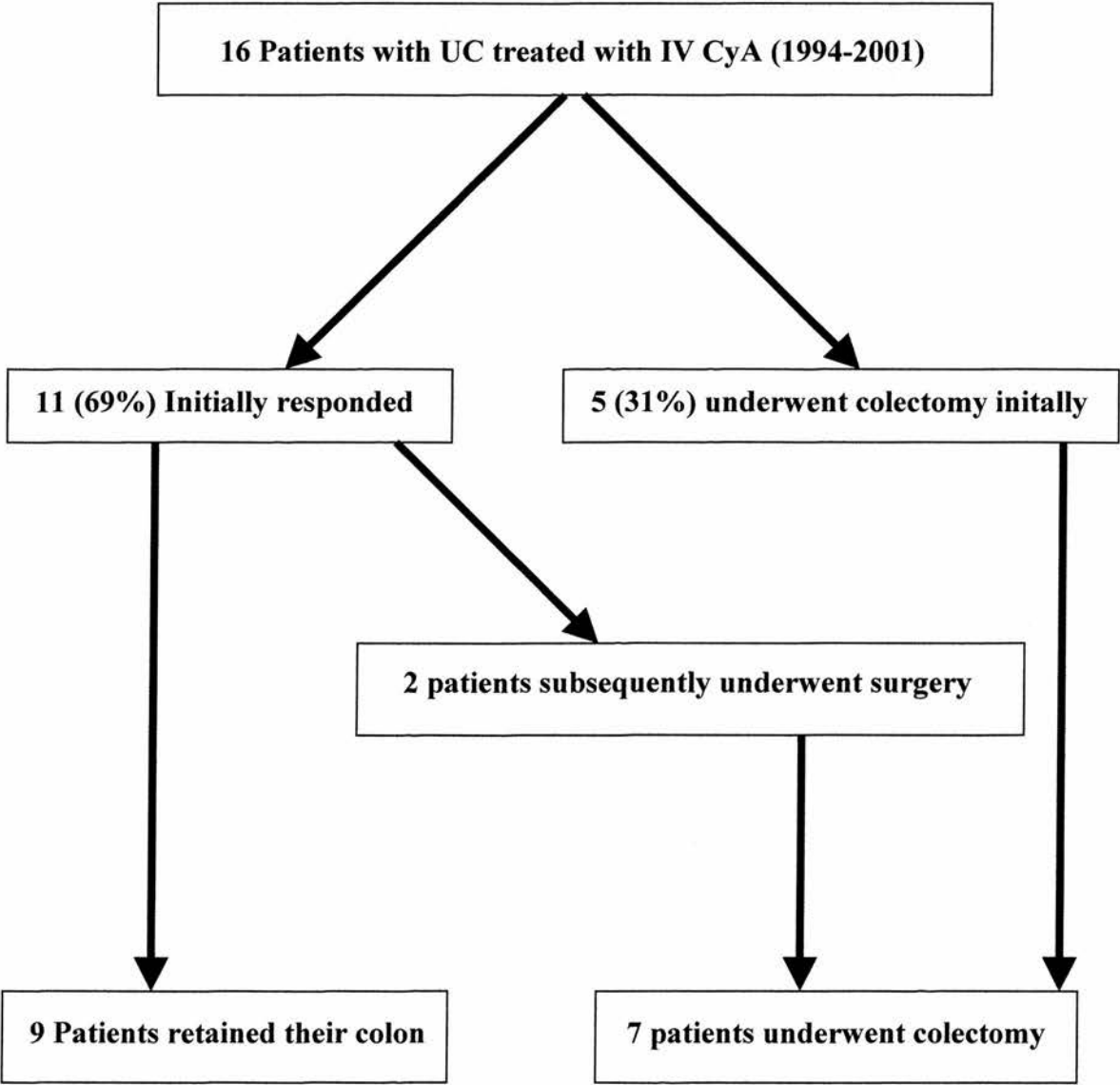


Table 7.3 – Patients who avoided surgery and duration of follow-up

Patient number	Duration of follow-up (months)
1	6
2	24
3	84
4	13
5	9
6	24
7	18
8	5
9	12

Chapter 8. White Cell Count at 4 months – a predictor of future myelosuppression in patients taking Azathioprine

8.1 Introduction

AZA use as a 2nd line immunosuppressant is well established. Approximately 10% of patients taking AZA have to discontinue it due to a variety of side effects, including myelosuppression.

There has been growing interest in the role of the enzyme TPMT in the development of neutropenia. Although there have been conflicting reports, it is generally accepted that low levels of TPMT are a risk factor for early bone marrow suppression in patients taking AZA (Colombel et al. 2000; Kader et al. 2000; Naughton et al. 1999; Sebbag et al. 2000; Stolk et al. 1998). There may be other factors that determine late-onset neutropenia in these patients such as viral infections such as Parvovirus B19 (Higashida et al. 1997).

Currently, TPMT activity monitoring and TPMT genotyping is not in widespread use in the UK. The use of more simple laboratory methods of predicting efficacy and toxicity have been examined, such as mean corpuscular volume (Decaux et al. 1999) and 6-thioguanine levels (Cuffari et al. 1996). We examined the nadir white cell count (WCC) at 4 months into AZA therapy in a cohort of IBD patients to see if this was a predictor of future myelosuppression in these patients.

8.2 Patients and Methods

The hypothesis was that WCC and neutrophil counts at 4 months do not correlate with IBD patients' respective nadir WCC and neutrophil count during AZA therapy.

We retrospectively examined our database of IBD patients who were currently taking AZA. They were a total of 172 patients (96CD:76UC, 76M:96F; median age 39 years, range 16-81 years) who were taking AZA for a minimum of 6 months and were stable of treatment (median duration of therapy 4.0 years, range 0.7-21 years). Information recorded was WCC at 4 months, neutrophil count at 4 months, nadir WCC and nadir neutrophil count during AZA therapy. In addition, AZA dose (mg/kg) and IBD disease (CD or UC) were recorded for these patients.

8.2.1 Statistical analysis - Regression analysis were performed on these data, correlating WCC and neutrophil count at 4 months with respective nadir WCC and neutrophil count during AZA therapy. Sub-group analysis for both CD and UC were also performed.

Correlation was assessed by calculating the Pearson correlation coefficient, a p value of <0.05 was regarded as significant. These were performed using Minitab v13.2 software.

8.3 Results

One hundred and seventy two IBD patients were evaluated (96CD:76UC, 75M:97F).

Mean AZA dose was 1.8mg/kg (SD=0.36) for this group. In the CD group (40M:56F), mean AZA dose was 1.75mg/kg (SD=0.22), and the UC group, mean AZA dose was 1.85 mg/kg (SD=0.40).

Regression analyses were performed for each patient group, comparing neutrophil count at 4 months against nadir neutrophil count and WCC at 4 months against nadir WCC. Figure 8.1 shows linear regression plot for IBD patients for neutrophil count and figure 8.2 WCC. Pearson coefficients were statistically significant for neutrophil count ($p<0.0001$), $r=0.70$ and WCC ($p<0.0001$), $r=0.69$. Figures 8.3 and 8.4 show the same analysis for CD patients. There was significant correlation for neutrophil count ($r=0.60$, $p<0.0001$) and WCC ($r=0.59$, $p<0.0001$). Finally, figures 8.5 and 8.6 show the same analysis for UC patients and they too were statistically significant (neutrophil count, $r=0.81$, $p<0.0001$; WCC, $r=0.80$, $p<0.0001$).

Figure 8.1 – Linear regression plot for Neutrophil counts for IBD patients

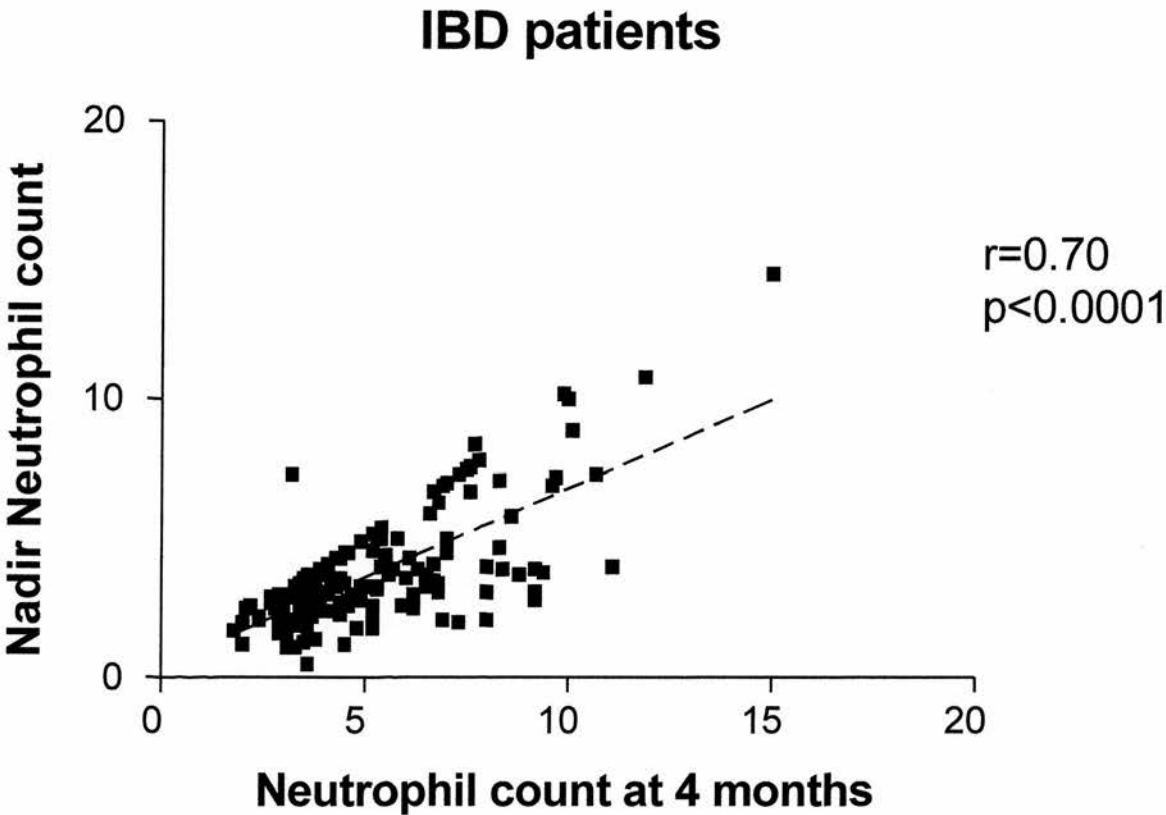
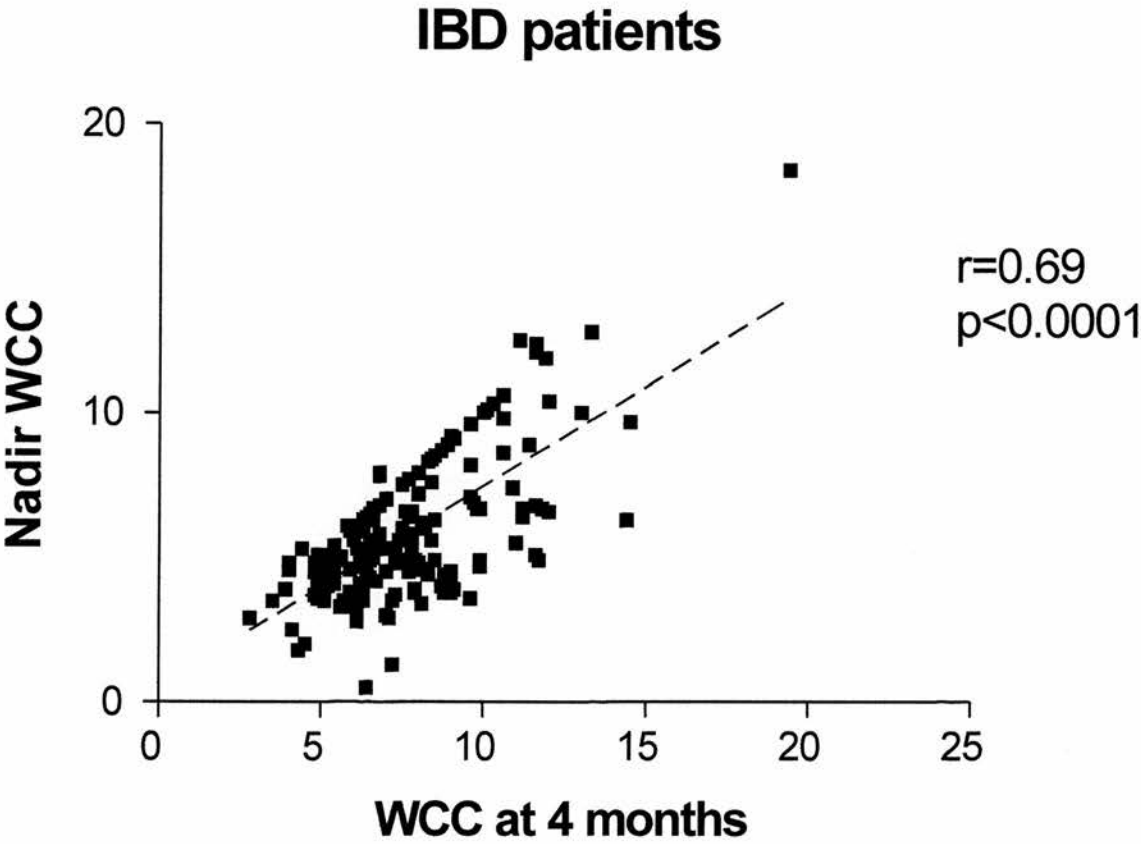


Figure 8.2 – Linear regression plot for WCC for IBD patients



**Figure 8.3 Linear regression plot for neutrophil count for Crohn’s
disease patients.**

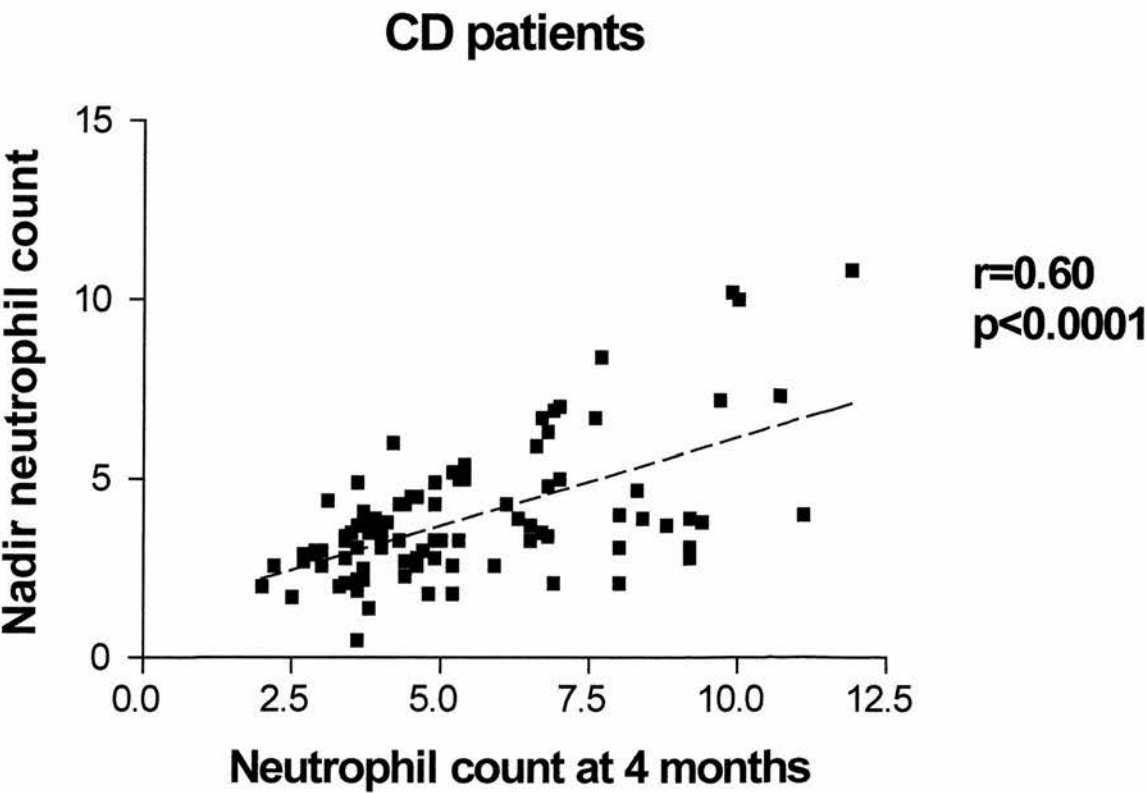


Figure 8.4 - Linear regression plot for WCC for Crohn's
disease patients.

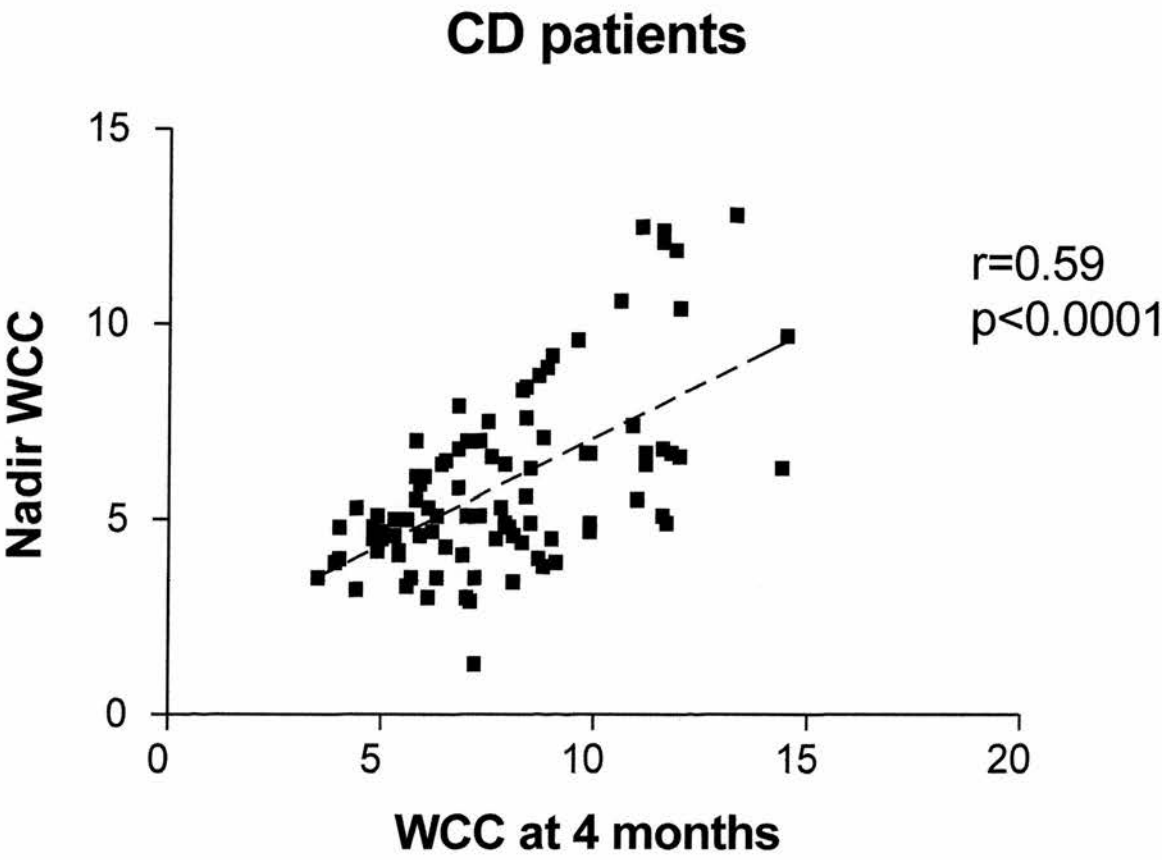


Figure 8.5 - Linear regression plot for neutrophil count in
ulcerative colitis patients.

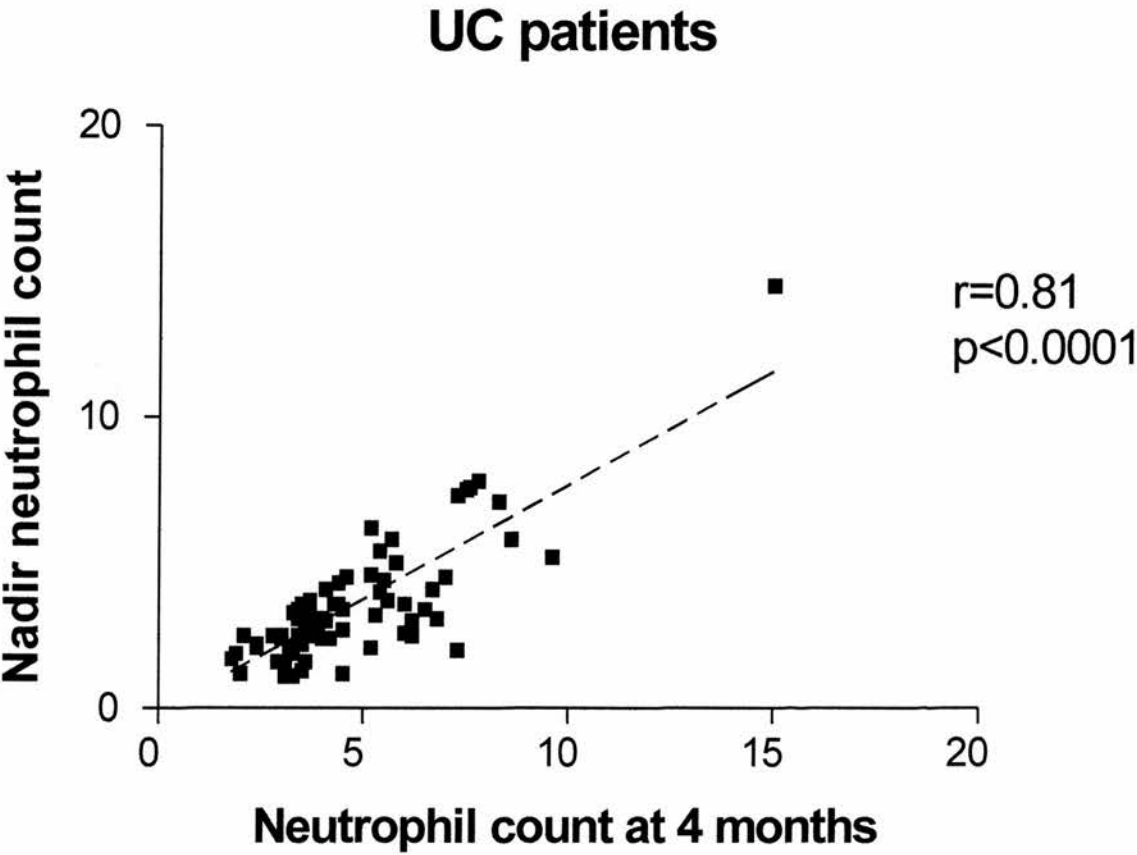
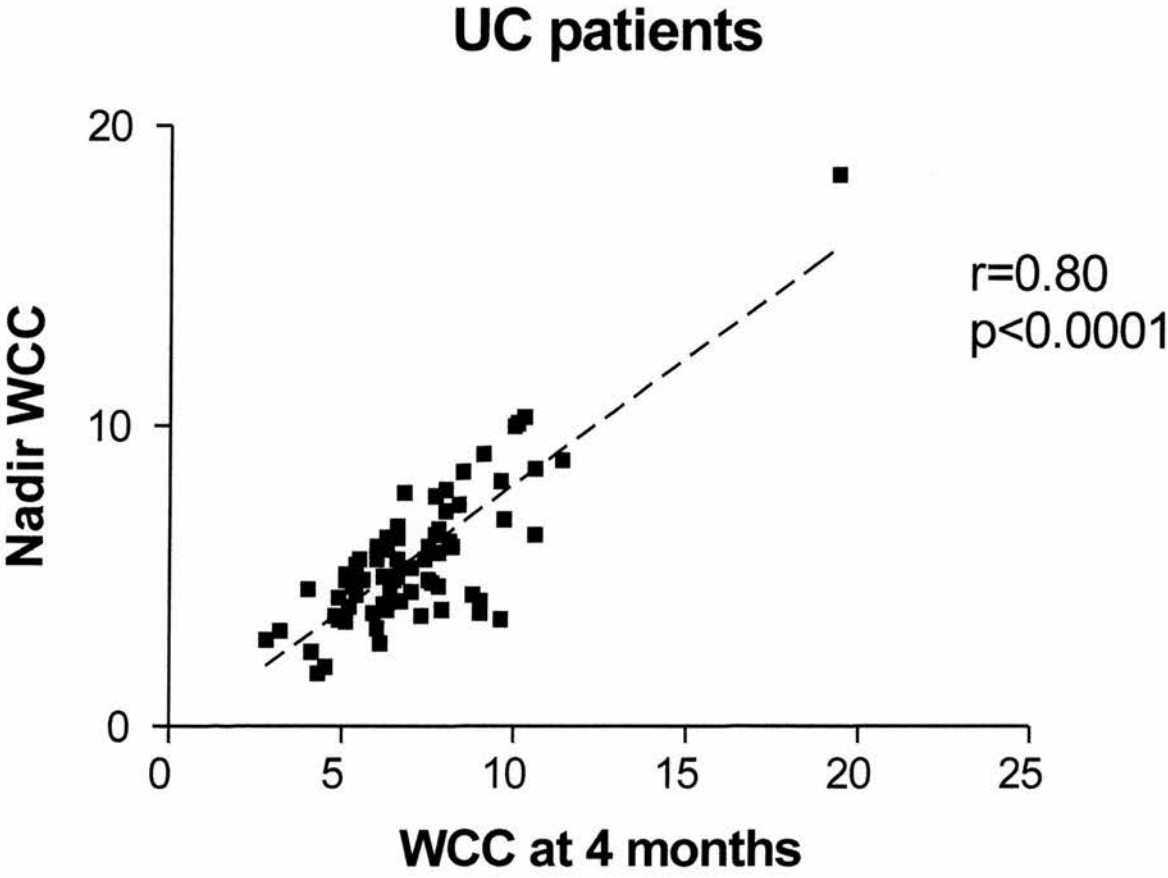


Figure 8.6 - Linear regression plot for WCC count in ulcerative colitis patients.



8.4 Discussion

There were 172 IBD patients who were taking AZA for a minimum of 6 months and were stable on AZA therapy. There was a statistically significant correlation between WCC and neutrophil count at 4 months and the lowest respective WCC and neutrophil count ever obtained during AZA therapy.

TPMT activity and genotype have been shown to correlate with risk of myelotoxicity. This has subsequently led to suggestions to introduce TPMT genotyping and or TPMT activity assessments in patients before they start on such immunosuppressive therapy (Jackson et al. 1997; Sebbag et al. 2000; Stolk et al. 1998). This is in the hope that this may reduce the need for regular blood count monitoring and prevent patients with genetically low levels of TPMT from potential life-threatening bone marrow suppression. However, there has been conflicting reports on the relevance of TPMT monitoring and bone marrow suppression (Kader et al. 2000; Naughton et al. 1999). It has been known for some time that bone marrow suppression can occur as a sporadic phenomena much later into AZA (Higashida et al. 1997) therapy and therefore the impact of introducing TPMT monitoring remains unknown, especially from a cost-benefit aspect (Tavadia et al. 2000). While some groups strongly advocate TPMT assessment prior to AZA therapy (Jackson et al. 1997; Lennard, 1998), others have shown that bone marrow suppression occurs at a higher frequency in the presence of normal TPMT levels than originally thought (Kader et al. 2000; Naughton et al. 1999) raised concerns that regular blood count monitoring should continue in these patients.

This data reveals a strong correlation between WCC/neutrophil count at 4 months and the nadir WCC/neutrophil count later on into AZA therapy. In this cohort, this data would support the idea that if bone marrow suppression is to occur, it will occur relatively early in therapy (as indicated by WCC/neutrophil count at 4 months). This would parallel that experience of our patients with low levels of TPMT taking AZA therapy.

Subsequent analysis of this data showed that MCV while on therapy with AZA or the change in MCV between pre-AZA values and while on stable AZA dosage did not correlate with either outcome as far as prevention of relapse was concerned or red cell TPMT activity. (Joy et al 2002)

In conclusion, WCC/neutrophil count at 4 months into AZA therapy appears to be a good predictor of future bone marrow suppression. It should be borne in mind that myelosuppression can occur at any time during AZA therapy and therefore, diligent blood count monitoring remains essential to the safety of patients taking such immunosuppressive therapy.

Chapter 9. Relevance of TPMT activity in IBD patients treated with low dose Azathioprine

9.1 Introduction

TPMT is a cytosolic enzyme that catalyses the S-methylation of aromatic and heterocyclical sulphydryl compounds including 6-MP and AZA. Metabolism of 6-MP is competitive between TPMT and two other enzymes (xanthine oxidase and hypoxanthine guanine phosphoribosyltransferase). TPMT converts 6-MP into an inactive form 6-methymercaptopurine while hypoxanthine guanine phosphoribosyltransferase converts 6-MP into its active form – 6-thioguanine (Kelley et al. 1967).

AZA is generally prescribed in a dose of 2mg/kg, though some authorities especially in the US, recommend higher doses of 2.5-3.0 mg/kg body weight. In the UK, it is not uncommon to prescribe doses lower than 2 mg/kg body weight, but the relationship of the various dosing regimens on effectiveness of maintenance with reference to TPMT activity has not been investigated.

Initial investigations into the clinical use and interpretation of intracellular measurements of TPMT activity were in children with acute lymphoblastic leukaemia. It has been known for some time that children with higher TPMT activities relapse more frequently than those with lower activity of this enzyme (Bostrom and Erdmann 1993; Lilleyman and Lennard, 1994). Induction of TPMT activity after

commencement of AZA/6-MP remains controversial, with conflicting reports in the literature(Keuzenkamp-Jansen et al. 1996; Weyer et al. 2001; Chocair et al. 1992). In addition, TPMT measurement during AZA therapy may be a more clinically relevant parameter than TPMT activity prior to commencement of AZA.

Momentum into investigating the implications of TPMT activity in IBD patients has steadily grown. In particular it is recognized that patients with low TPMT activity are more susceptible in developing bone marrow suppression side effects (Dubinsky et al. 2000; Lennard, 1998; Stolk et al. 1998). The impact of TPMT activity on the clinical course of IBD patients treated with low dose AZA has not been studied to date.

In this study we aimed to retrospectively evaluate the clinical course of IBD patients currently taking AZA and relate this to their respective TPMT activity.

9.2 Materials and Methods

The hypothesis was that there is no difference in remission maintenance in IBD patients treated with AZA when comparing low TPMT activity (<20nmol/hour/ml) versus normal TPMT activity (>20nmol/hour/ml).

9.2.1 Patients

We recruited a cohort of 113 IBD patients (52UC:61CD, 57 female:56 male, median age = 45 years, range 18-71 years) who were taking AZA, had discontinued AZA because of side effects or who had never taken AZA. We compared these patients with a group of 17 healthy controls (8 female:9 male, median age = 39 years, range 26-62 years). Median duration of follow up was 4.7 years (range 0.6-9.9 years). TPMT activity was determined from blood samples by a radiochemical assay detailed below. Relapse rates per year of follow up (number of relapses divided by years of treatment) and time to first relapse were analysed and compared with their respective TPMT activity and their current AZA dose per kilogram body weight. TPMT activity was classified as low if activity was below 20 nmol/h/ml RBC. All cases were examined by one investigator (SC).

Other information that was collected included the lowest neutrophil count ever recorded during therapy, concomitant drug therapy, anatomical disease distribution, AZA dose and patients body weight at induction of therapy. Relapse was defined by necessity for surgery, endoscopic criteria or clinical recurrence (as assessed by an experienced clinician based on clinical features and inflammatory parameters - ESR, CRP, white cell count and platelets). Surgery for purely mechanical obstruction,

without abnormal inflammatory parameters was not considered a relapse of active disease. Endoscopic findings complemented clinical features and blood parameters, but were not used solely to determine relapse.

9.2.2 Blood Samples

Blood was collected by venepuncture into 2.7ml EDTA vacutainer tubes. Red blood cells (RBC) were washed twice with pH 7.4 phosphate buffer solution (PBS) and then centrifuged at 3000 rpm for 10 minutes.). Haematocrit of a suspension of 0.5mls RBC suspended in 0.5 mls PBS were then measured. Finally, the suspension was haemolysed with 2.8mls of ice-cold distilled water and 0.7mls aliquots were pipetted into 1.5ml epindorff tubes ready for the assay.

9.2.3 TPMT assay

TPMT activity was measured in haemolysed red blood cells utilising the following reaction :-



Haemolysed red cells were spun at 13000 rpm for 10 minutes in a microfuge centrifuge at 4°C. For each sample, 100µl red cell lysate were then added to 15ml plastic tubes for blank and the test estimation (both in duplicate). Twenty-five microlitres of pH 7.5 potassium phosphate buffer was added to each tube, then 5ul of

6-MP was added to the test and 5µl of dimethylsulphoxide (DMSO) added to the other

2. The reaction was started by adding 25µl of reagent mixture (containing ^{14}C -S-adenosyl methionine, S-adenosyl methionine and dithiothreitol solution) and incubating at 37°C in a shaking water bath for 1 hour. The reaction was stopped by the addition of 500µl of 0.5M borate buffer pH10.0. The methyl-mercaptopurine was extracted into an isoamyl alcohol/toluene mixture and centrifuged at 2700rpm for 10 minutes at room temperature. One point five millilitres of the organic phase was then added to 10mls scintillant (OpticSafe, Pharmacia) and radioactivity counted for each of the vials for 10 minutes in a β -scintillation counter. The activity of TPMT in the RBC samples was calculated from the concentration of ^{14}C -methyl mercaptopurine in the test sample (corrected for the blank sample) and the activity expressed as nmol/hour/ml RBC.

9.2.4 Statistical analysis

Relapse rates per year of follow up were compared in patients with their TPMT activity by non-parametric Mann-Whitney test (MWT) and p values of ≤ 0.05 were regarded as significant. Times to first relapse were also compared by Kaplan-Meier curves using log rank analysis and Chi square testing. Comparisons between multiple groups were performed using ANOVA analysis, $p \leq 0.05$ was considered significant. All statistical analyses were performed using Minitab (release 13.20) statistical software.

9.3 Results

One hundred and thirty blood samples were analysed from 113 IBD patients and 17 healthy controls. The IBD patients were categorized as 'intolerant', 'maintenance azathioprine' or as 'azathioprine naïve' (see table 9.1). The 'intolerant' group were patients who had previously received AZA, but had subsequently discontinued treatment because of side effects (pancreatitis, bone marrow suppression, abdominal pain, nausea, dermatological manifestations). The 'maintenance azathioprine' group were patients who were currently taking and stable on AZA therapy. The 'azathioprine naïve' group were IBD patients who had never received AZA treatment.

Figure 9.1 illustrates TPMT activity in these groups; analysis comparing the control group against the other 3 groups was not significant by MWT and shown in the figure.

The 'intolerant' category was then further analysed. Side effects were individually grouped as neutropenia (neutrophil count $<2.5 \times 10^6$ - mean TPMT 19nmol/hr/ml), pancreatitis (as defined by clinical symptoms and a raised amylase- mean TPMT 24 nmol/hr/ml), hepatitis (raised alanine transaminase twice above baseline measurement – mean TPMT 28nmol/hr/ml), dermatological (skin rashes that resolved once AZA was stopped – mean TPMT 27 nmol/hr/ml) and other (a variety of side effects including non-specific abdominal pain, nausea, vomiting, taste disturbance and headaches – mean TPMT 27 nmol/hr/ml). This is shown in figure 9.2.

The mean TPMT activity in the neutropenic patient group was significantly lower than the mean TPMT activity of patients who had other side effects (ANOVA, $p < 0.05$).

Table 9.1 - Patient category characteristics

	N=	Male:Female	Median age – years (range)	Median AZA dose – mg/kg (range)	Median duration AZA – years (range)	Median TPMT activity nmol/hr/ml (range)	Concurrent 5-ASA	Crohn’s disease Terminal ileum – TI Ileocolonic – IC Colonic - C	Ulcerative colitis Pan – P Left sided – L Distal – D
Controls	17	8:9	39 (26-62)	N/a	N/a	29 (20-35)	Nil	N/a	N/a
AZA Intolerant	24	11:13	39 (21-66)	1.4 (0.6-2.3)	0.9 (0.1-4.6)	25 (14-41)	13	13 TI- 3 IC- 5 C- 5	11 P- 5 L- 4 D- 2
AZA Maintenance	63	33:40	44 (18-69)	1.75 (1.4-2.4)	3.0 (0.6-9.9)	24 (9-35)	35	33 TI- 4 IC- 11 C- 18	30 P- 10 L- 11 D- 9
AZA Naïve	26	13:13	48 (21-71)	N/a	N/a	24 (14-40)	16	15 TI- 6 IC- 4 C- 5	11 P- 2 L- 4 D- 5

FIGURE 9.1 - TPMT activity plotted against subgroups of IBD and controls (AZA intolerant n=24, AZA maintenance n=63, AZA naïve n=26, controls n=17)

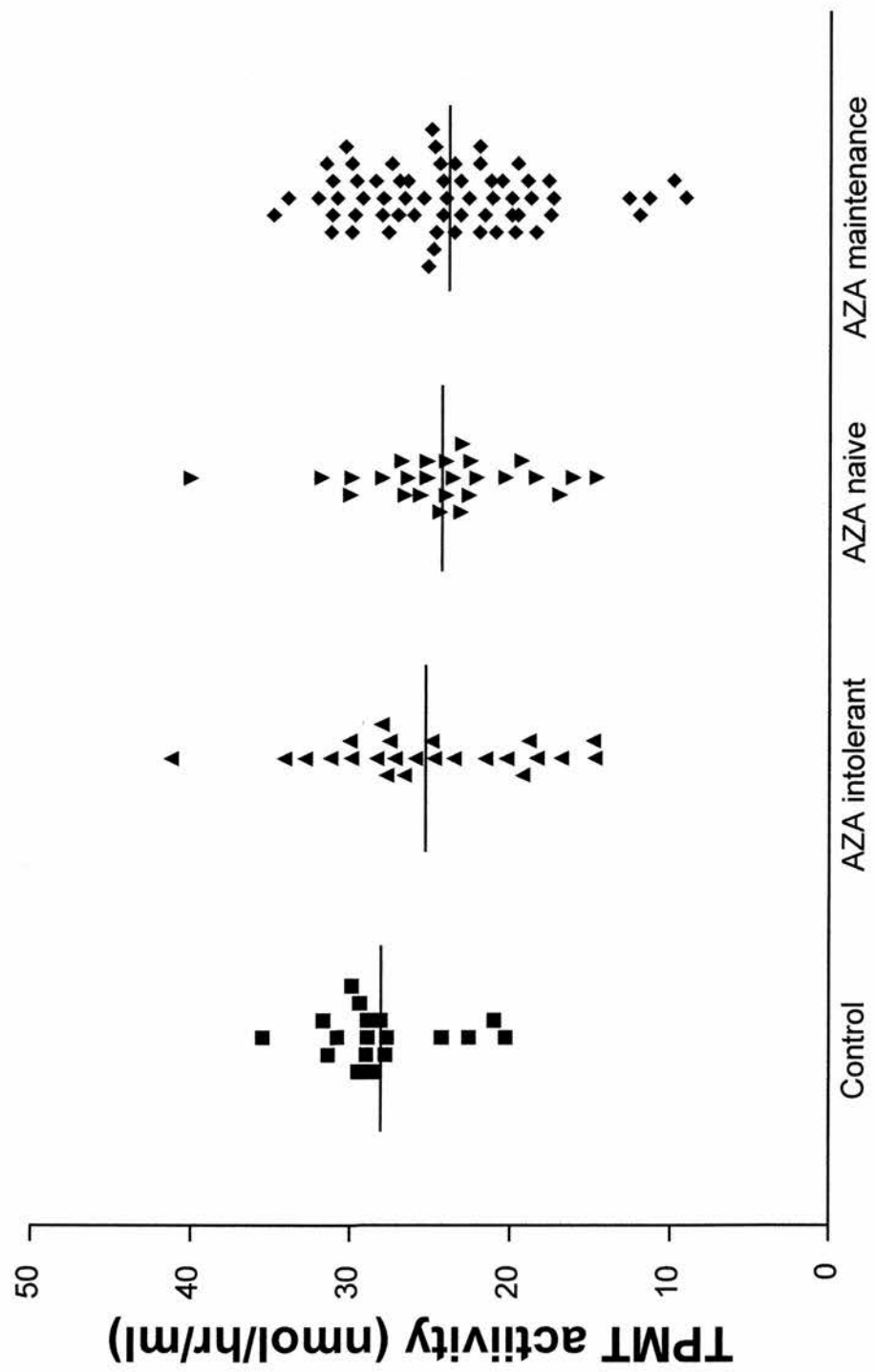
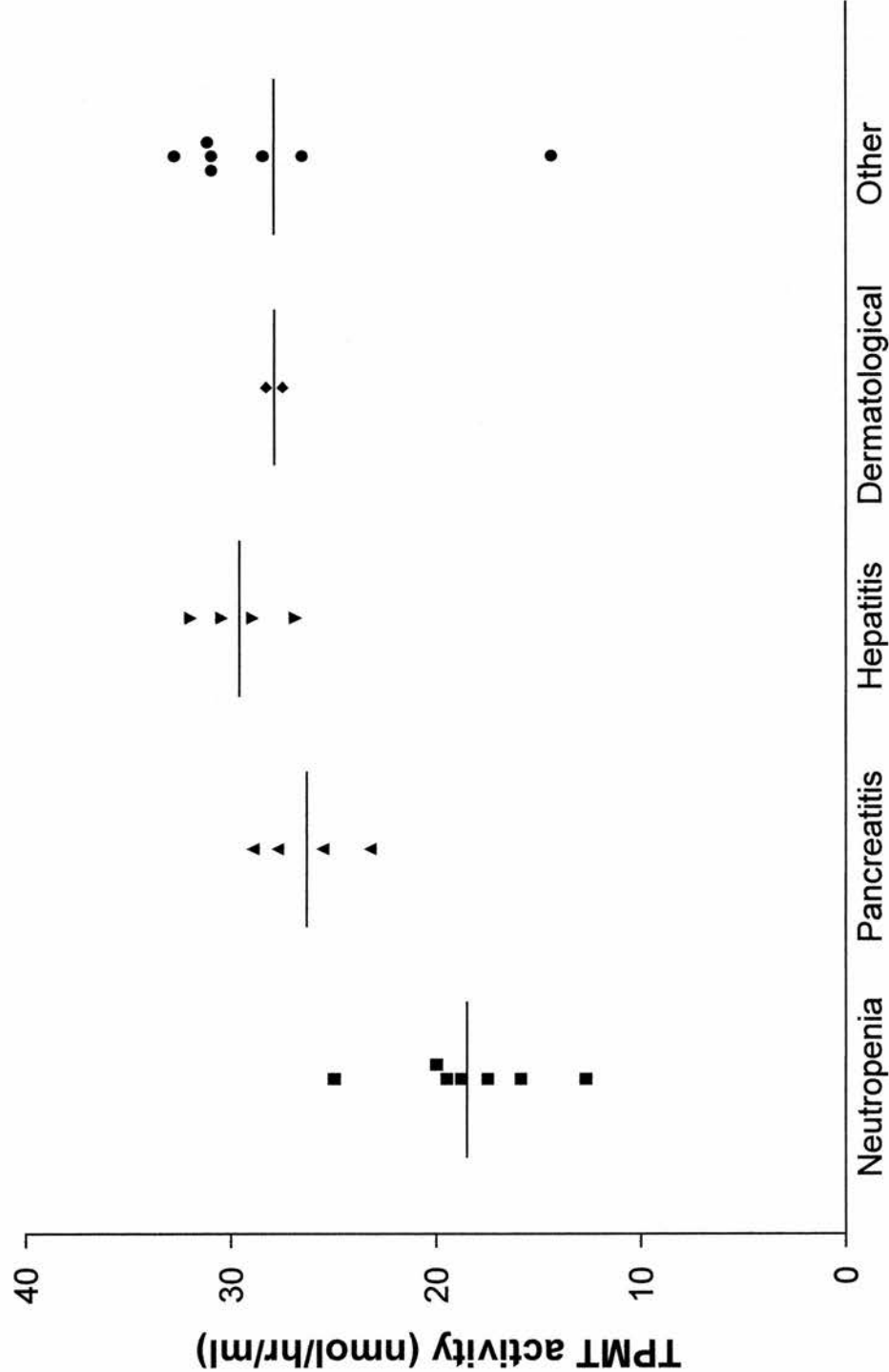


FIGURE 9.2- TPMT activity in respective side effect groups (neutropenia n=7, pancreatitis n=4, hepatitis n=4, dermatological n=2, other n=7). The horizontal bars represent mean TPMT activity. TPMT values in the neutropenic group were significantly lower than other groups of side effects (ANOVA, $p<0.05$)



To investigate the impact of TPMT activity upon relapse rates in IBD patients we examined 2 groups. Both groups were taking AZA at a dose of less than 2mg/kg for a minimum of 1 year. The groups were subdivided by their respective relapse rates as either 'non-relapsers' (n=20) or 'relapsers' (n=14) (i.e. more than zero relapse per year of follow up). These groups were well matched for AZA dose (1.5mg/kg vs. 1.45 mg/kg), duration of follow up (3.0 years vs. 2.6 years) and age (36 years vs. 35 years). The mean TPMT activity within the 'non-relapser' group was 19.8 nmol/hr/ml versus 27.6 nmol/hr/ml for the 'relapser' group. This was statistically significant (Mann-Whitney Test, $p < 0.005$). These results are summarised in table 9.2. To further analyse this a Kaplan-Meier survival curve was constructed based on time to first relapse in the *low-dose* AZA treated patients (< 2.0 mg/kg body weight, n=34, 19CD:15UC) for TPMT activity of < 20 nmol/hour/ml and > 20 nmol/hour/ml (Figure 9.3). Log rank analysis was statistically significant ($\chi^2 = 4.0$, $p < 0.05$). Further survival curves were constructed for CD only (Figure 9.4) and UC only patients (Figure 9.5), and log rank analysis was significant in both groups (for CD, $\chi^2 = 3.2$, $p = 0.04$ and for UC, $\chi^2 = 2.9$, $p = 0.05$).

Finally the 'maintenance azathioprine' group was divided into low TPMT activity (using the lower limit of our control group – < 20 nmol/hr/ml) or normal TPMT activity (> 20 nmol/hr/ml) and constructed further survival curves based on time to first relapse for *all* IBD patients. There were a total of 63 patients taking AZA (33 female:30 male, 33CD:30 UC, median age 44 years). Mean follow up in this group was 3.0 years with a median AZA dose of 1.75 mg/kg (range 1.4-2.4 mg/kg). This is shown in Figure 9.6. Log rank analysis did not show statistical significance ($\chi^2 = 0.2$, $p = \text{NS}$) despite a trend of shorter time to relapse in patients with TPMT activity

Table 9.2 – Comparison of low dose AZA (<2mg/kg) patients

	N=	Female :Male	Median Age – years (range)	Median AZA dose – mg/kg (range)	Median AZA duration – years (range)	Concurrent 5-ASA	Median duration of AZA – years (range)	Crohn’s disease Terminal ileum – TI Ileocolonic – IC Colonic -C	Ulcerative colitis Pan – P L side – L Distal – D	Mean TPMT activity (nmol/hr/ml)	Mann- Whitney Test (p=)
Low dose – non- relapsers	20	12:8	36 (21-72)	1.50 (1-1.8)	3.0 (1.1-11.8)	11	0	TI – 3 IC – 3 C – 6	P- 3 L – 3 D – 2	19.8	P<0.0005
Low dose – Relapsers	14	7:7	35 (24-52)	1.45 (1.2-1.8)	2.6 (1.8-2.9)	6	0.5	TI – 2 IC – 3 C – 2	P – 2 L – 2 D – 3	27.6	

FIGURE 9.3 - Time to first relapse for low dose AZA (<2mg/kg) IBD patients (n= 34) according to TPMT

activity

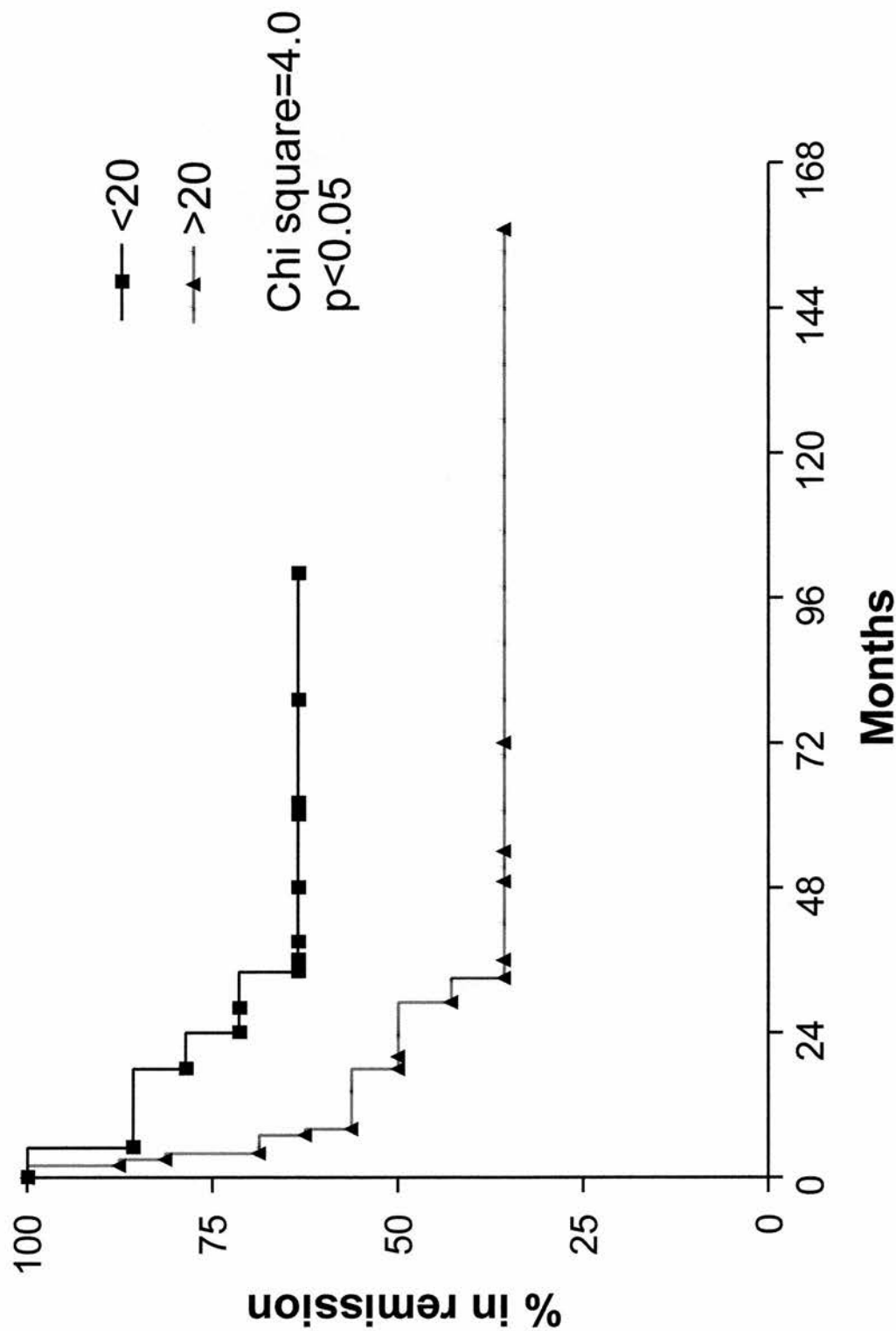


FIGURE 9.4. – Time to first relapse for low dose AZA (<2mg/kg) CD patients (n= 19) according to TPMT activity

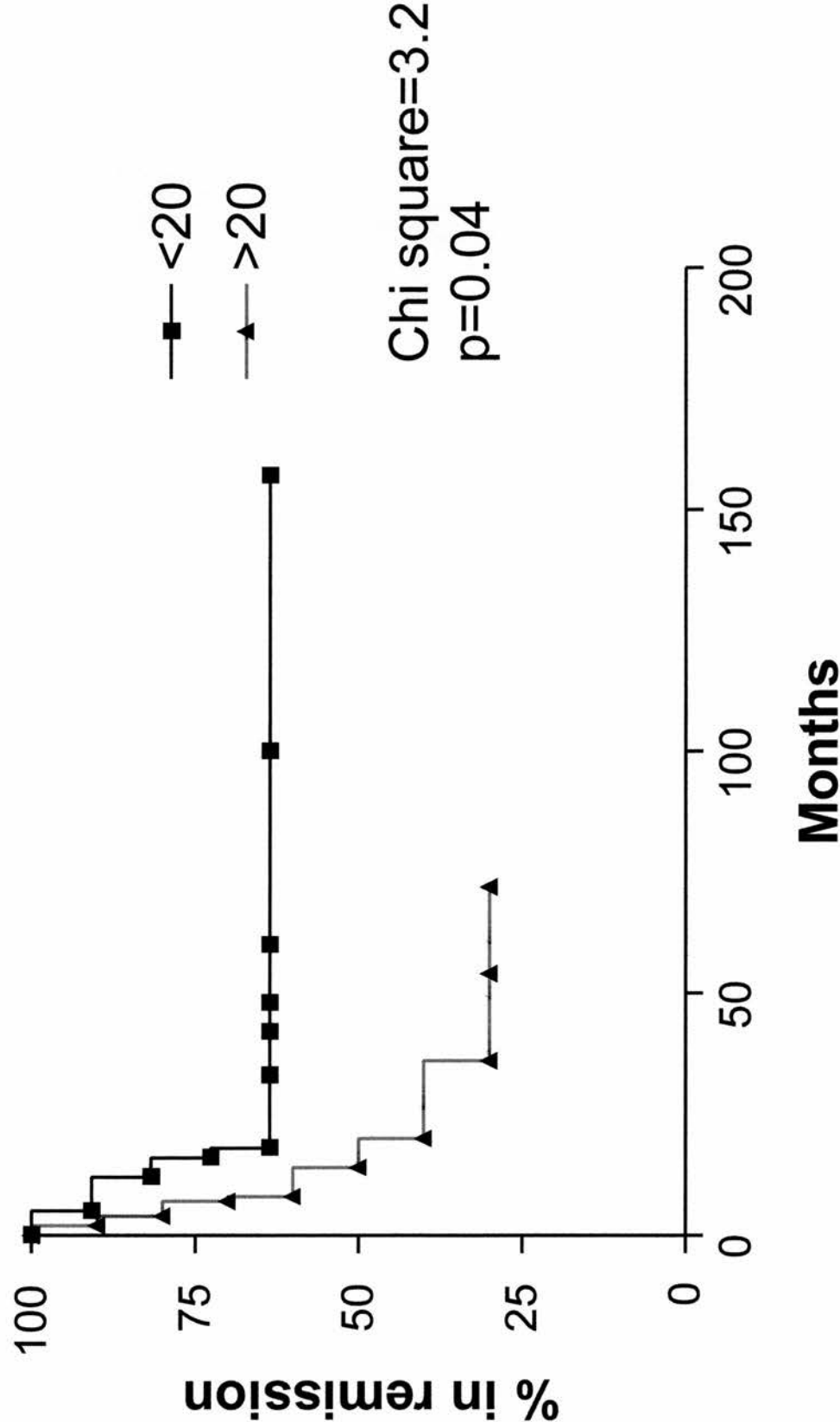


FIGURE 9.5 - Time to first relapse for low dose AZA (<2mg/kg) UC patients (n=15) according to TPMT activity

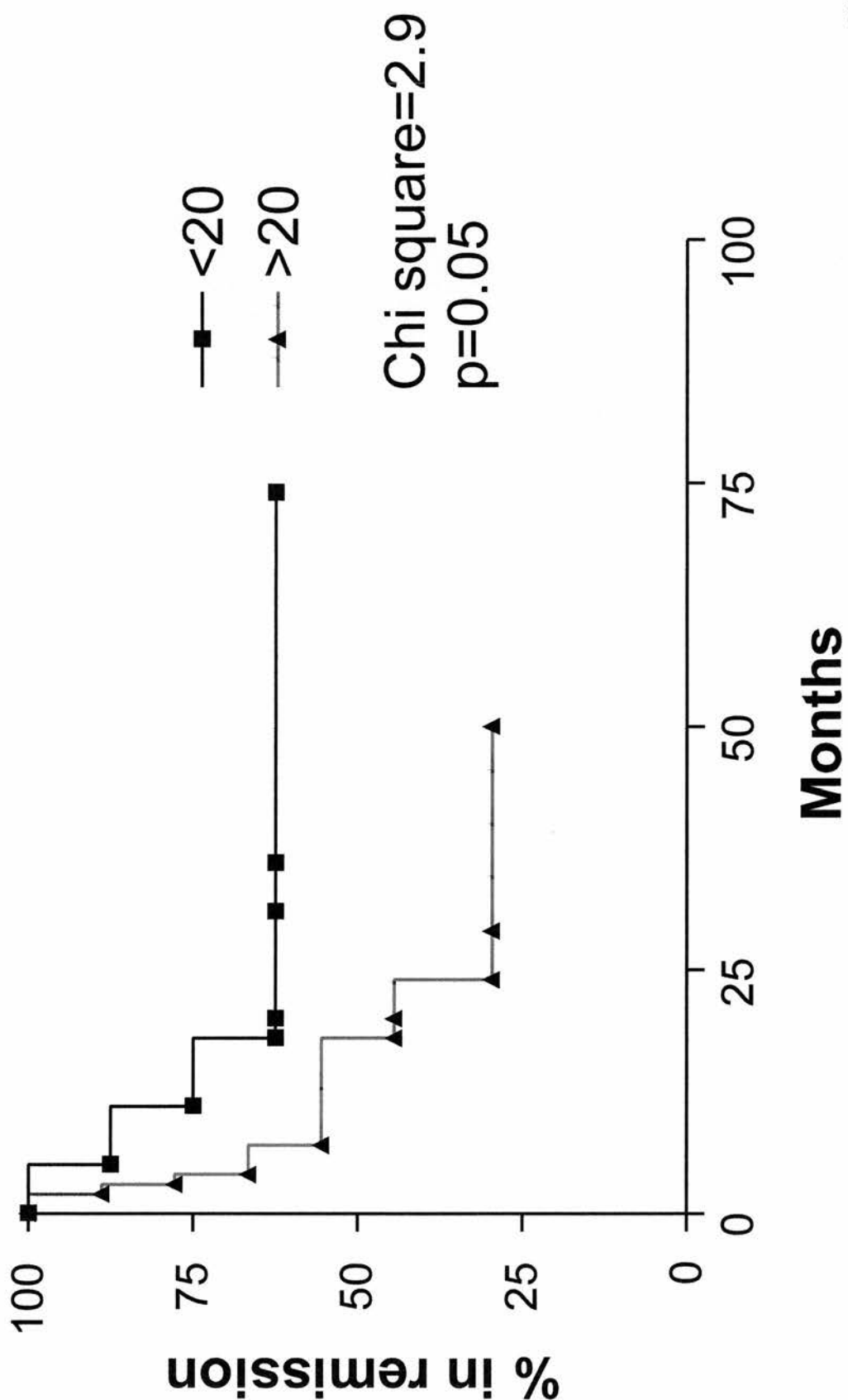
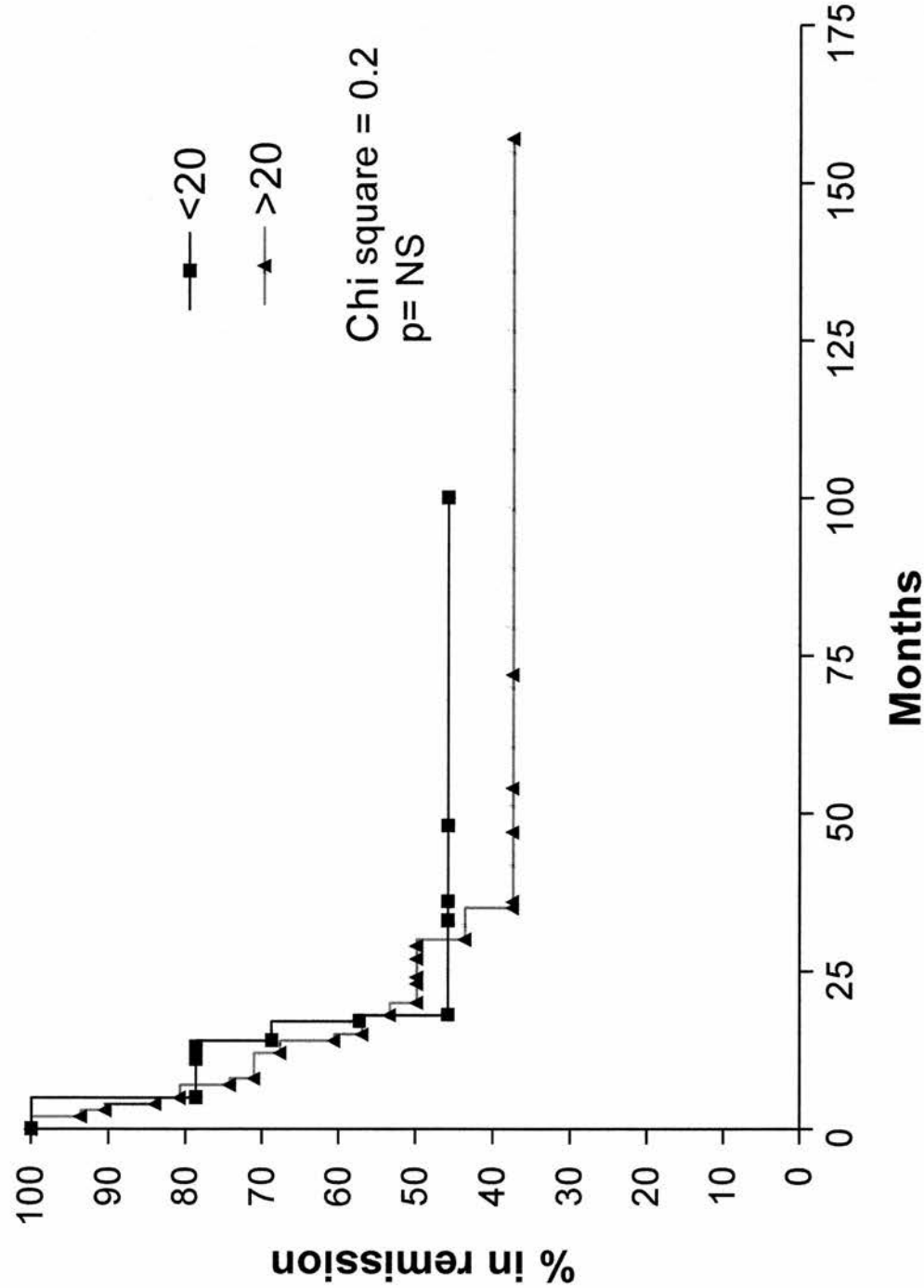


FIGURE 9.6 - Survival curve for all IBD patients (n=63) on AZA according to TPMT activity



>20nmol/hr/ml. Further analyses were performed on CD only (Figure 9.7) and UC groups only (Figure 9.8) - Log rank analysis did not show statistical significance in either group (CD, $\chi^2=0.22$, p=NS and CD, $\chi^2=0.06$, p=NS).

FIGURE 9.7 - Survival curve for all CD patients (n=33) on AZA according to TPMT activity

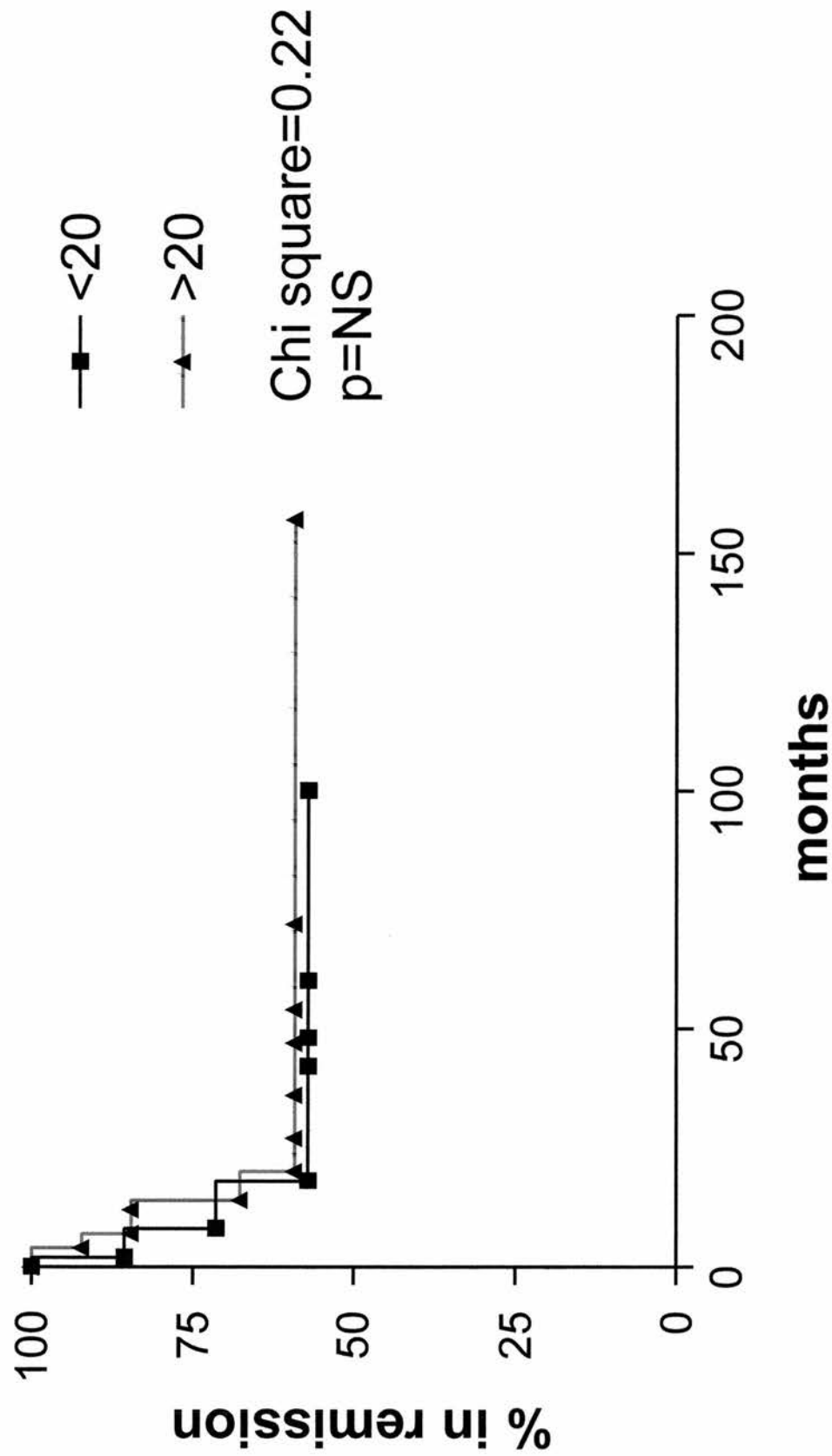
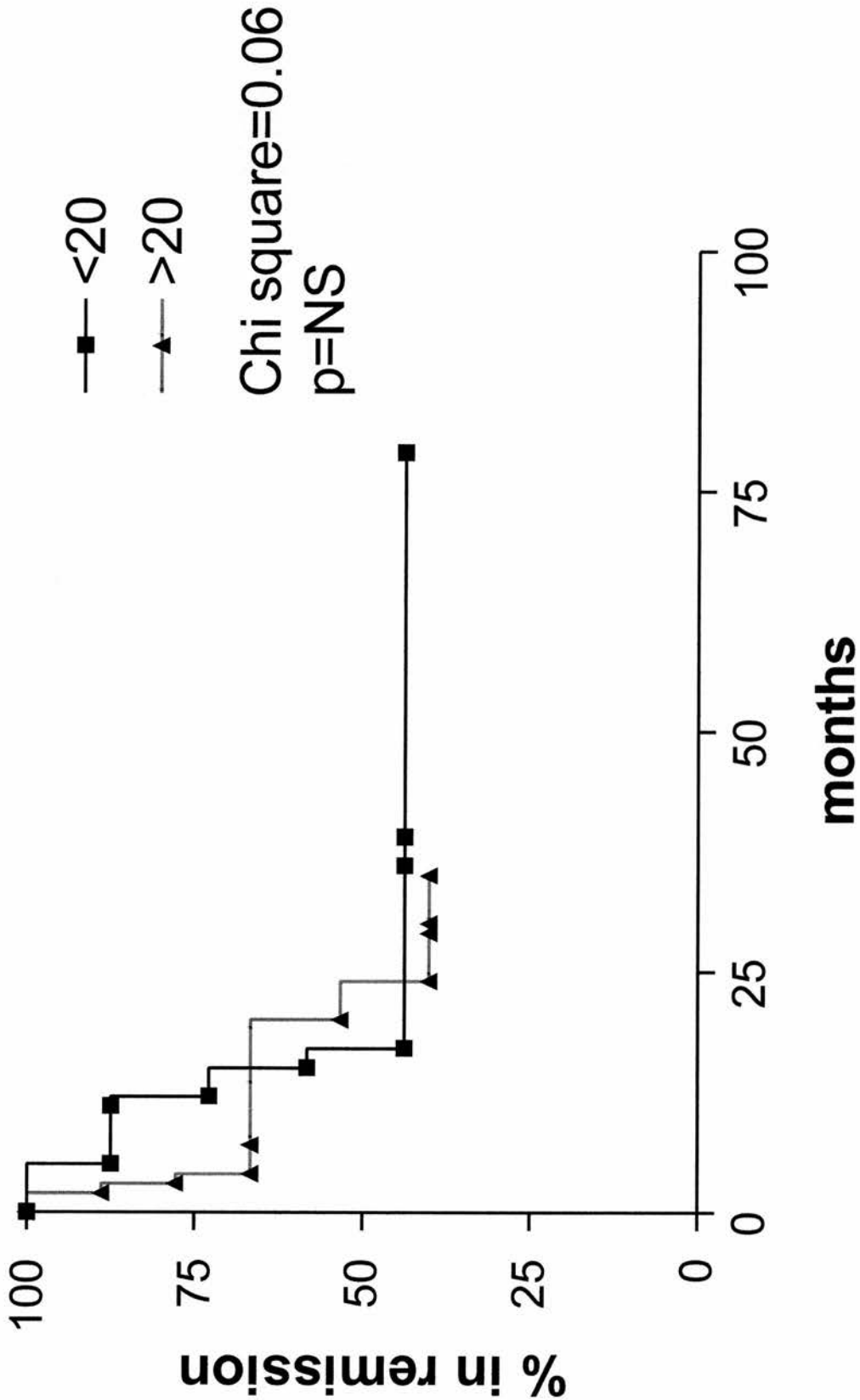


FIGURE 9.8 - Survival curve for all UC patients (n=30) on AZA according to TPMT activity



9.4 Discussion

Low TPMT activity appeared to be predictive of a favourable response in patients treated with lower doses of AZA (< 2mg/kg). None of the patients in this cohort had extremely low TPMT activity (<5nmol/hr/ml) suggesting that none were homozygote recessive for the TPMT gene (Weyer et al 2001). As shown by other studies, there is an inverse relationship between red cell TPMT activity and 6-TG levels in acute lymphoblastic leukaemia - corresponding to a reduce risk of relapse but a higher probability of neutropenia (Bostrom and Erdmann 1993; Lilleyman and Lennard, 1994). This relation with neutropenia was also confirmed in this study. TPMT activity correlated well with lowest neutrophil count within the first 4 months of AZA therapy (figure 9.9) in this group ($r=0.41$, $p=0.0001$). It should be noted that patients who became neutropenic within the first 4 months maintained this degree of neutropenia throughout AZA therapy. In this study there was no difference in time to first relapse in *all* IBD patients on AZA with low (<20 nmol/hr/ml) or normal (>20 nmol/hr/ml) TPMT activity (figure 9.6), further confirming that neutropenia is not required for disease remission maintenance. Over the past two decades, it has become clear that while AZA remains an essential second line agent in the treatment of steroid resistant or refractory IBD, its relatively narrow therapeutic index means that patients may not fully benefit from a 'standard dose' of AZA (Weinshilboum and Sladek, 1980).

TPMT activity is determined by a polymorphic gene in a co-dominant fashion leading to a trimodal distribution of phenotypes (Lennard et al. 1987) and there also evidence of ethnic variation of TPMT activity (Kumagai et al. 2001; McLeod et al. 1994; McLeod et al. 1999). Genotype TPMT^L/TPMT^L gives rise to low TPMT

activity and accounts for only 1% of the western population (McLeod et al. 1994; Weinshilboum and Sladek, 1980) - these patients are particularly susceptible to serious bone marrow suppression side effects (Dubinsky et al. 2000). Although our cohort did not have any patients with a phenotype like this, it may be possible that these homozygote recessive patients could be safely treated with AZA – but at very low doses (<0.5mg/kg). Conversely, genotype TPMT^H/TPMT^H (approximately 9% of the western population (McLeod et al. 1994; Weinshilboum and Sladek, 1980)) leads to high TPMT activity and hence these may benefit from higher doses (>2mg/kg) of AZA that are not currently routinely prescribed in the UK. The majority of the population (90%) are heterozygotes TPMT^H/TPMT^L giving a wide spectrum of TPMT activity (McLeod et al. 1994; Weinshilboum and Sladek, 1980). It is this majority of patients that would also further benefit by dose titration according to their TPMT activity, as shown by this study. There have also been concerns in paediatric patients treated with 6-MP that low levels of TPMT may have secondary carcinogenic effects on bone marrow stem cells in leukaemia patients receiving chemotherapy protocols including 6-MP (Thomsen et al. 1999). This clearly needs to be borne in mind, especially when contemplating long term AZA therapy. However, it remains unknown whether ‘dose adjustment’ for lower TPMT activity may have the benefit of reducing such complications in the long term.

The principal finding of this study is that if a low dose regimen of AZA i.e. <2mg/kg body weight is used, knowledge of the TPMT phenotype is essential. If a low dose of AZA is used in IBD patients with a high TPMT phenotype, the number of relapses suffered is likely to be high and maintenance of remission imperfect. The higher doses of AZA generally recommended and used in the US probably saturates this

phenotypic variation in TPMT activity, resulting in higher overall remission rates. Though speculative, it is possible that the higher dose of AZA is only necessary in patients with higher TPMT activity. Our study results also provide an explanation for the commonly observed phenomenon of prolonged remissions on a low dose of AZA in a proportion of UK IBD patients. In future it may be possible to take into account the phenotypic variation of TPMT in deciding on the exact dosage of AZA to be used in an individual patient.

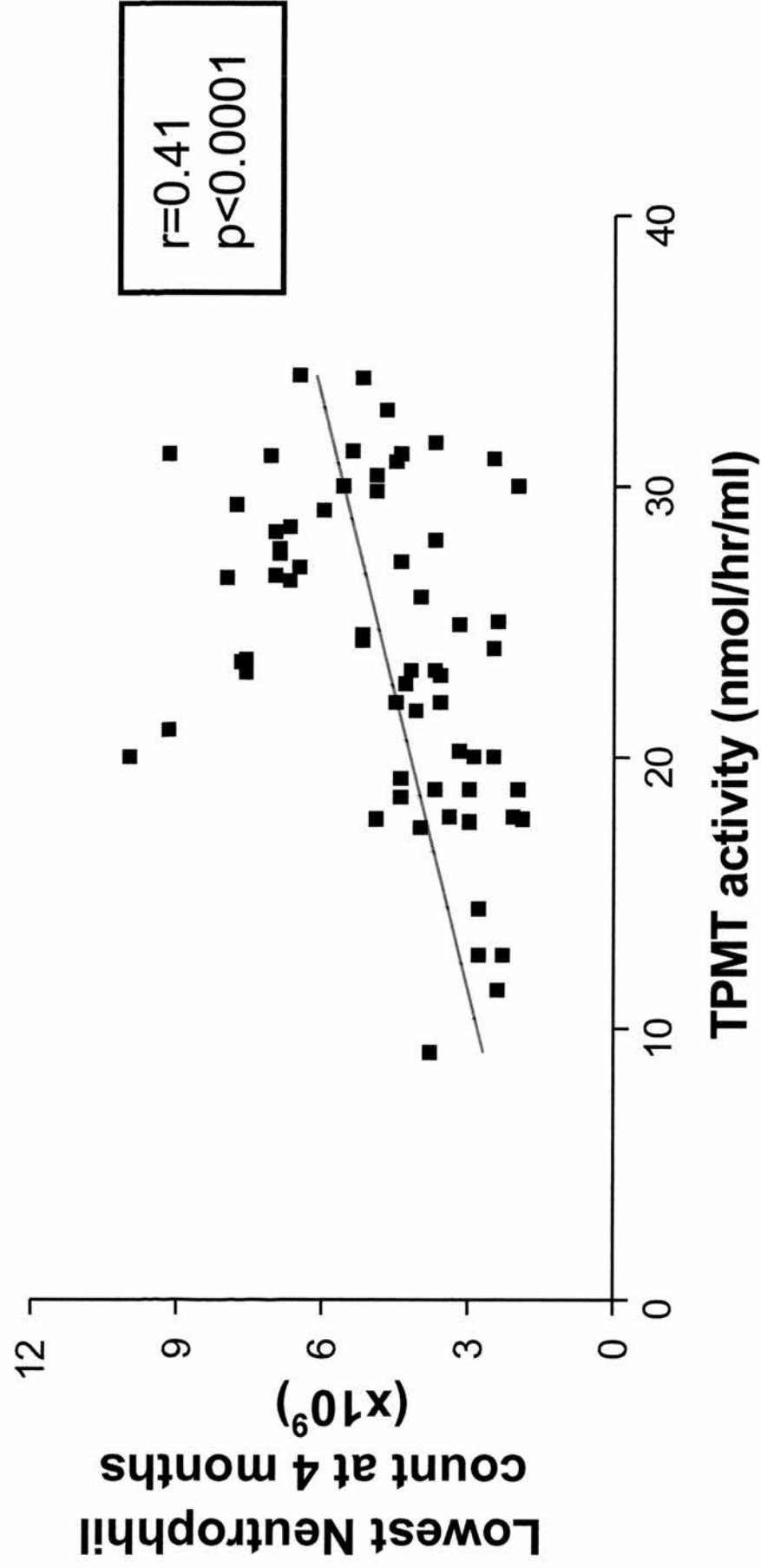
This study used data from a large database of IBD patients who were well matched for disease type and distribution, and there were comparable numbers of patients taking 5-aminosalicylate (5-ASA) compounds to those who were not (table 2). However, this is a retrospective analysis of patients and therefore subject to bias. In addition, patients TPMT activity was measured while they were on concurrent medication that may have induced or inhibited red cell TPMT activity. However, this was addressed by the fact that groups were well matched with regards to their concomitant medication type. Induction of TPMT activity by AZA remains a controversial issue. Studies reporting significant rises in TPMT activity after AZA have predominantly been in renal transplant patients(Weyer et al. 2001; Chocair et al. 1992; Cho et al. 2000) and leukaemia patients(Lennard et al. 1987; Lennard et al. 1990). These patients have been started on other concomitant medications that may also be responsible for TPMT induction in their own right, an observation reported previously with other commonly prescribed medications (Lennard, 1998). Furthermore, it has recently been shown that uraemia in renal transplant is a significant inducer of TPMT activity, making the role of AZA in the induction of TPMT less clear (Weyer et al. 2001). Only one study to date has addressed the issue of TPMT activity induction in

healthy controls following administration of 6-MP, and they reported no induction of TPMT activity (Keuzenkamp-Jansen et al. 1996). Finally, it is worthy to note that since some have reported somewhat erratic levels of TPMT activity induction following commencement of AZA (McLeod et al. 1995), it could be argued that a single TPMT activity measurement while stable on AZA therapy is more clinically relevant. To date, there have been no studies addressing these issues in IBD patients. Further analysis were also performed on patients taking 5-ASA medications, in view of recent concerns over in-vitro 5-ASA enzyme inhibition (Lewis et al. 1997; Lowry et al. 1999; Szumlanski and Weinshilboum, 1995). Mean TPMT activities in IBD patients either taking 5-ASA versus those who were not were not statistically significant (data not shown).

This is the first report addressing the issue of TPMT activity and disease relapse in IBD patients to the author's knowledge, opening up a further avenue in enabling the physician to individualize effective AZA therapy in these patients. We have shown that in patients on a low dose of AZA (<2mg/kg), the mean TPMT activity was significantly lower in those in remission compared with those that relapsed during the follow-up period. TPMT activity was significantly lower in patients who discontinued AZA due to neutropenia compared to TPMT activity of patients who discontinued AZA due to other side effects.

The limitations of a retrospective study and potential selection bias of patients are acknowledged, and prospective studies to tailor AZA dosage to TPMT polymorphism are required.

Figure 9.9 – Regression plot of nadir neutrophil count against TPMT activity



Chapter 10. Final Discussion

AZA immunosuppression is probably mediated through its effects on lymphocytes, although there appears to be preferential suppression of neutrophils when leucopenia occurs (Korelitz et al. 1997). This suggests that the anti-inflammatory action of AZA is mediated through its effects on neutrophils. Indeed, it has been shown that neutrophil migration into the lamina propria occurs during clinical relapse in IBD (Teahon and Bjarnason 1993). Recent data also suggests that lymphocyte apoptosis may also underlie the action of AZA (Tiede et al 2002).

From the cohort of 173 patients studied, remission maintenance was not statistically different in neutropenic versus non-neutropenic patients. Even when a stricter cut off for neutropenia and analysis were performed (using neutrophil count $\leq 2 \times 10^9$) there was no significant differences in relapse rates. The lowest neutrophil count reflected in general the pattern of neutrophil counts closely. Analysis of the lowest neutrophil counts within the first 4 months of therapy correlated well with the nadir neutrophil count (Pearson correlation coefficient $r = 0.7$, $P < 0.005$), supporting that neutropenia is not purely a sporadic phenomenon. Fraser et al (Fraser et al 2002) examined a larger cohort of IBD patients ($n = 424$) retrospectively. They found that a lower mean WCC (mean WCC 5.86×10^9 in remission achieved group versus mean WCC 7.91×10^9 in remission not achieved, $p = 0.0001$) and lower mean neutrophil count (mean neutrophil counts were 3.83×10^9 in remission achieved group versus 5.85×10^9 in remission not achieved group, $p = 0.0001$) were a predictive factor for achieving remission. Remission induction with respect to neutrophil count was not analysed in our data. Further comparison were made on remission maintenance according to the nadir

WCC during treatment – patients with a WCC of $<5.0 \times 10^9$ were more likely to maintain remission than those with higher nadir WCC, $p=0.03$. However, their data did not examine the nadir neutrophil count as studied here. It is difficult to draw any comparative conclusions with our data, especially when lymphopenia (a common phenomenon) may have contributed to some degree to their overall WCC. Work by Colonna et al (Collona and Korelitz 1994) also examined leucopenia in 98 CD patients. They concluded that patients achieving leucopenia were more successful in maintaining clinical remission. This study's main weakness is in the small patient numbers, which limits the power of the study and the short duration of follow-up (18 months). They also did not examine neutrophil count.

Dose titration of AZA to achieve neutropenia cannot be justified from our results. The study involves a large number of patients ($n=173$) over a long follow-up period of up to 21 years. Although the retrospective nature of the study is a weakness, the power of this analysis is sufficient to detect a clinically relevant difference in remission maintenance. It could be argued that the potential risks of neutropenia alone would require at least this clinically relevant difference before neutropenia could be used as a goal of AZA treatment in IBD. Finally, a prospective trial looking into dose titration and degree of neutropenia is probably of too high a patient risk to be practical to perform.

There was a statistically significant correlation between WCC and neutrophil count at 4 months and the lowest respective WCC and neutrophil count ever obtained during AZA therapy. TPMT activity and genotype have been shown to correlate with risk of myelotoxicity. This has given hope that the introduction of TPMT genotyping and or TPMT activity assessments in patients before they start on such immunosuppressive

therapy(Jackson et al. 1997; Sebbag et al. 2000; Stolk et al. 1998) may reduce the need for regular blood count monitoring. There have been conflicting reports on the relevance of TPMT monitoring and bone marrow suppression (Kader et al. 2000; Naughton et al. 1999), and it has been know for some time that bone marrow suppression can occur as a sporadic phenomena much later into AZA (Higashida et al. 1997) therapy. In addition, bone marrow suppression appears to occur at a higher frequency in the presence of normal TPMT levels than originally thought (Kader et al. 2000; Naughton et al. 1999) raising concerns that regular blood count monitoring should continue in these patients. In this cohort, the data would support the idea that if bone marrow suppression is to occur, it will occur relatively early in therapy (as represented by nadir WCC/neutrophil count at 4 months). It would seem sensible that as long as the potential for myelosuppression at any time during is borne in mind, the nadir WCC/neutrophil count can be used as a cost effective predictor of patients who are more likely to require either cautious dose escalation or early cessation of AZA. Remission maintenance (as defined as relapse rates per year of follow up) were not statistically different between the AZA + 5ASA groups and the AZA alone group (in UC or CD groups). The cumulative remission curves compare well with other studies which have looked at remission maintenance with 5-ASA and AZA(Messori et al, 1994; Pearson et al. 1995; .d'Albasio et al, 1997; Gendre et al, 1993; Rijk et al. 1992; Bouhnik et al, 1996). From our study, it was not possible to elucidate the impact of 5-ASA compounds on long-term malignancy risks in these patients because of its retrospective nature. Although there are recent studies advocating the protective role of 5-ASA drugs in the development of colorectal malignancy (Davis et al. 1992; Ekbohm, 1995; Moody et al. 1996; Andrianopoulos et al. 1989; Eaden et al. 2000), it still remains uncertain whether this is a direct effect of 5-ASA drugs or merely as a

result of prolonged disease remission. There was no obvious excess occurrence of malignancy in our group of patients taking 5-ASA, although some have advocated the use of folate supplementation to reduce the incidence of dysplasia and CRC in chronic UC patients maintained on sulphasalazine (Lashner et al 1989).

Despite recent reports of a potentially important drug interaction between 5-ASA compounds and TPMT raising concerns over increased susceptibility of neutropenia (Lowry et al 1999, Lewis et al 1997). The results from our data do not support the idea that this is clinically relevant based upon discontinuation rates in the AZA+5-ASA group compared to AZA alone. This is further supported by work from Lowry et al (Lowry et al 2001) who found that the WCC in patients taking AZA+5-ASA was significantly lower than patients on AZA alone, albeit that the WCC remained within normal limits.

It would therefore appear that 5-ASA compounds afford no additional benefit to AZA as judged by remission maintenance. Although this is perhaps not unexpected in CD patients, this may help rationalise drug treatment in UC patients taking AZA+5-ASA. This study used data from a large database (n=172) that were particularly well matched for disease type and distribution. In addition, comparable numbers of patients were taking 5-ASA drugs as were not. Although it could be argued that patients taking AZA alone had more aggressive disease and hence bypassed 5-ASA therapy, this is not support from the standpoint of previous relapses and anatomical extent of disease. To answer questions over the potential role of malignancy/dysplasia protection, the role of the TPMT/5-ASA interaction and further characterise the exact place of 5-ASA therapy a prospective, double blinded, placebo control trial now needs to be performed. However the duration of follow-up (a minimum of 3 years) and large

patient numbers (more than 200) that would be required to give some of these answers. This could be done through a multicentre study.

In humans, the effect of AZA on pregnancy and offspring has been predominantly studied in organ transplant patients - usually taking a combination of other immunosuppressants including steroids, CyA or tacrolimus. Nonetheless cytogenetic analysis has detected chromosomal aberrations in these offspring, but long term follow up has not revealed further problems in these infants (Willoughby et al. 1971). In addition, there is no current evidence of fertility problems in patients taking AZA (Thiersch 1962). Immunological abnormalities have also been reported in the newborn's of these patients (Golby, 1970; Williamson and Karp, 1981), constituting lymphopenia, thymic hypoplasia and bone marrow hypoplasia, but all of these have subsequently resolved later in infancy. Animal studies using much higher doses of AZA (20mg/kg!) have shown teratogenicity (Gross et al. 1977, Alstead et al 1990), but it is difficult to extrapolate these experiences into humans, especially when AZA handling has been shown to be different in these animals.

In this study, 2 offspring suffered congenital abnormalities. There were no skeletal deformities recorded in any of the infants (as reported anecdotally by others (Schwartz 1966)), and it would seem more plausible that these infants had sporadic abnormalities considering their polygenic aetiology in the normal population. Previous studies in groups of IBD patients taking AZA have also recorded healthy offspring, although there have been cases of viral infections - Hepatitis B and Cytomegalovirus in the mother and subsequently the offspring (Saarikoski and Seppala, 1972; Willoughby et al. 1971).

This study has several weaknesses. Namely its small numbers and retrospective nature, as well as a lack of detailed immunological information on the offspring – something that could only be obtained in a prospective setting. While we should be cautious whenever using AZA during pregnancy, a more pragmatic approach should be sort after full discussion with mother and father. Currently there seems to be a small risk of prematurity and or possibly congenital abnormalities, but this has to be balanced by the effects of sub-optimal disease control (in the mother) and its effect on potential offspring. The study highlights all the problems about studying such a small niche of patients – especially when there are such differing clinical practices across the world. In addition, it highlights the difficulty in performing a prospective randomised study in pregnant women – the issue of equipoise. Equipoise – the state of mind characterized by the legitimate uncertainty as to choice of treatment strategy due to a balance of benefits versus risks. While equipoise would be achievable in the treating physician, it is highly unlikely that the same could be achieved in the protective mother-to-be. This latter problem makes any potential prospective study unlikely to be able to recruit sufficient numbers of patients to give enough power to answer the hypothesis that AZA therapy does not affect pregnancy outcome in IBD patients. It should also be borne in mind that this represents the largest study into AZA use in pregnant IBD patients in the last decade and compares well with the outcomes found by Alstead et al in 1990 (Alstead et al 1990). What is needed for the future is a multicentre study using a specifically set up national database, so that further conclusions can be drawn about the use and safety of AZA on offspring of IBD patients.

Even less attention has focussed upon the progeny of fathers who have received similar AZA therapy. There have been several small papers describing experiences pregnancies in the partners of male renal transplant patients – more than 90% of births were overtly normal, with spontaneous abortion, neural tube defects and trisomy 21 making up the remainder of birth complications (Eslami et al. 1976; Papoff et al. 1977; Whetham et al. 1983). To date there have been no published studies in IBD patients taking AZA.

In this cohort, there were 2 progeny who later developed complications – one autism and the other a cerebral astrocytoma at the age of 10 years. These 2 conditions are relatively common childhood illnesses and it would be difficult to argue that they were attributed by AZA therapy. While the aetiology of autism is unknown, there have been reports of associations with maternal hypothyroidism (Gillberg et al. 1992). Cerebral tumours are the most common childhood cancers and their incidence in the UK is rising (McKinney et al. 1998). The design of this study did not allow for a detailed evaluation of fertility issues, but there were no reports of problems with partners conceiving from the questionnaire. Other studies of spermatogenesis in humans have had conflicting results with both normal and low sperm quality being reported (Evans et al. 1975, Salant et al. 1976). Other studies in patients with rheumatoid arthritis (Currey et al. 1974) and systemic lupus erythematosus (Masramon et al. 1980) revealed normal sperm counts in all of their cohorts. Again, there is scope for further research in this area. A prospective study with sperm analysis as well as more detailed progeny analysis is now required to look at the effects of AZA in IBD male patients prior to their partner's conception.

CyA rescue therapy has previously been shown to be extremely useful in inducing remission and saving patients with UC from surgery. Early response rates have been reported to be between 60 to 80% (Lichtiger and Present 1990; van Gossum et al. 1996; Sandborn 1995). Our series also confirms this relatively high initial response rate (11 patients, 69%). Our long-term success rate was also comparable to that found by other studies (Sandborn 1995). Only 2 (22%) patients who were started on concurrent AZA later required surgery, with the remaining 7 patients maintaining clinical remission to date (and also exhibited a significant steroid sparing effect). This further advocates the concurrent use of oral AZA in the setting of CyA, is much more impressive than the colectomy rate previously published with CyA therapy without subsequent oral AZA (Gurudu et al. 1999). Side effects are a significant cause of morbidity in patients treated with CyA (Cohen RD et al. 1999). Again, our experience was similar to that published previously. More importantly though is the fact that none of these side effects were deemed severe enough by the patient or physician to merit discontinuation which may have been aided by strict and regular CyA level monitoring and dose adjustment in this group.

Finally, this study supports the value of predictive markers of surgery previously reported by Travis et al (Travis et al 1996) – namely bowel frequency and CRP at day 3 (albeit that the latter did not reach statistical significance).

This CyA study contains only a small number of patients and was performed in a retrospective manner. However, its importance is shown by the higher proportion of patients taking AZA as a long-term maintenance following remission induction by IV CyA. The fact that these patients were from a single centre and therefore subject to uniform clinical practice strengthens the fact that discontinuation of CyA because of

side effects was not encountered here. Clearly a larger prospective study is required to place AZA as a useful adjunct to CyA rescue therapy. Randomisation between CyA + oral AZA + oral steroid against CyA and oral steroid therapy with a significant follow up time of 18 months would help to prove or refute its usefulness. So far this data adds further evidence for CyA as a 3rd line drug therapy in patients where surgery is undesirable.

My final chapter examines the interesting field of pharmacogenetics in AZA use. Over the past two decades, it has become clear that AZA is an essential second line agent in the treatment of steroid resistant or refractory IBD, its relatively narrow therapeutic index means that patients may not fully benefit from a 'standard dose' of AZA (Weinshilboum and Sladek, 1980). TPMT activity has been shown to be an important predictor of myelotoxicity in patients taking AZA. Indeed my work on TPMT confirmed a reproducible method by which TPMT activity can be determined from a simple blood sample. My results confirmed work by others that a lower activity of TPMT correlated well with the neutrophil count and low TPMT activity was a predictor for discontinuation of AZA due to neutropenia rather than other side effects (Bostrom and Erdmann 1993; Lilleyman and Lennard, 1994). The polymorphic gene that dictates TPMT activity is well documented. There is also evidence of ethnic variation of TPMT activity (Kumagai et al. 2001; McLeod et al. 1994; McLeod et al. 1999), suggesting that the TPMT phenotype may be a more clinically relevant parameter when optimising AZA therapy. This work confirms that importance, since the majority of patients who are heterozygote TPMT^H/TPMT^L have a wide range of TPMT activities (McLeod et al. 1994). The study confirms for the first time that IBD patients on lower AZA doses (<2mg/kg) and lower TPMT activity

levels maintain remission longer than patients with normal/high TPMT activity levels. These results provide an explanation for a commonly observed phenomenon of prolonged remissions in patients on relatively low doses of AZA. In addition, this opens further potentially exciting avenues for tailoring individuals AZA therapy – it may be possible for example to safely treat patients who have very low TPMT activity (i.e. TPMT^L/TPMT^L genotype) with ultra-low doses of AZA and the converse may also hold true.

This study used data from a large database of IBD patients who were well matched for disease type and distribution, and there were comparable numbers of patients taking 5-ASA compounds to those who were not. However this is a retrospective analysis and therefore subject to bias. In addition, patients TPMT activity was measured while they were on concurrent medication, which may have induced or inhibited red cell TPMT activity. The latter point is an important one. While this remains a controversial issue, the majority of studies to date have reported somewhat erratic levels of TPMT activity induction following commencement of AZA (McLeod et al 1995). It can therefore be argued that a single measurement of TPMT while on AZA is a more clinically relevant parameter by which to adjust drug doses etc. To help answer this question more comprehensively a prospective study is now needed, measuring TPMT activity pre and post AZA as well as correlating 6-TG antimetabolite levels with these measurements and matching patient groups for concurrent drug types and doses. The search for the optimal formula for AZA therapy in IBD continues.

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Relevance of thiopurine methyltransferase activity in inflammatory bowel disease patients maintained on low-dose azathioprine. Campbell et al. APT, 2002. 16(3): 389-398

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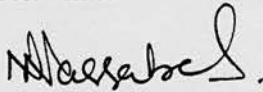
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Relevance of thiopurine methyltransferase activity in inflammatory bowel disease patients maintained on low-dose azathioprine

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SUMMARY

Background: It is well-recognized that patients with low thiopurine methyltransferase activity are more susceptible to the development of bone marrow suppression side-effects.

Aim: To study the impact of thiopurine methyltransferase activity on the clinical course of inflammatory bowel disease patients treated with low-dose azathioprine (< 2 mg/kg).

Methods: We measured the thiopurine methyltransferase activity of blood samples from 113 inflammatory bowel disease patients who were taking azathioprine, had discontinued azathioprine because of side-effects, or had never taken azathioprine. The thiopurine methyltransferase activity was compared with that of 17 healthy controls. Relapse rates and time to first relapse were compared in inflammatory bowel disease patients and stratified according to their thiopurine methyltransferase activity.

Results: Patients who became neutropenic had a significantly lower mean thiopurine methyltransferase activity than that of patients who developed other side-effects (analysis of variance, $P < 0.05$). Survival curves were constructed (time to first relapse) for patients treated with low-dose azathioprine for thiopurine methyltransferase activities of < 20 and > 20 nmol/mL red blood cells/h. There was a significantly lower number of relapses in inflammatory bowel disease patients with lower thiopurine methyltransferase levels ($P < 0.05$).

Conclusions: The mean thiopurine methyltransferase activity was significantly lower in patients on a low dose of azathioprine in remission compared with those who relapsed. The thiopurine methyltransferase activity was significantly lower in patients who discontinued azathioprine due to neutropenia than in those who discontinued due to other side-effects.

INTRODUCTION

Thiopurine methyltransferase (TPMT) is a cytosolic enzyme that catalyses the S-methylation of aromatic and heterocyclic sulphydryl compounds, including 6-mercaptopurine and azathioprine. The metabolism of 6-mercaptopurine is competitive between TPMT and two other enzymes (xanthine oxidase and hypoxanthine

guanine phosphoribosyltransferase). TPMT converts 6-mercaptopurine into an inactive form, 6-methylmercaptopurine, while hypoxanthine guanine phosphoribosyltransferase converts 6-mercaptopurine into an active form, 6-thioguanine¹ (Figure 1).

Azathioprine is generally prescribed in a dose of 2 mg/kg, although some authorities, especially in the USA, recommend higher doses of 2.5–3.0 mg/kg body weight. In the UK, it is not uncommon to prescribe doses lower than 2 mg/kg body weight, but the relationship of the various dosing regimens to the effectiveness of maintenance with reference to TPMT activity has not been investigated.

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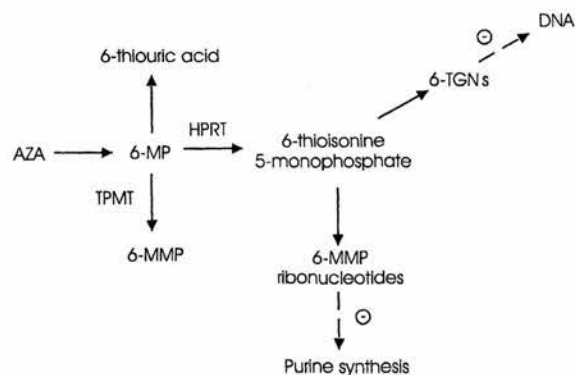


Figure 1. Mechanism of azathioprine metabolism: AZA, azathioprine; HPRT, hypoxanthine guanine phosphoribosyltransferase; 6-MMP, 6-methylmercaptopurine; 6-MP, 6-mercaptopurine; 6-TGN, 6-thioguanine; TPMT, thiopurine methyltransferase.

It is well recognized that patients with low TPMT activity are more susceptible to the development of bone marrow suppression side-effects.^{2, 3} Initial investigations into the clinical use and interpretation of intracellular measurements of TPMT activity were in children with acute lymphoblastic leukaemia. It has been known for some time that children with higher TPMT activities relapse more frequently than those with a lower activity of this enzyme.^{4, 5} The induction of TPMT activity after commencement of azathioprine/6-mercaptopurine remains controversial, with conflicting reports in the literature.^{6–8} Such induction is variable and unpredictable. In addition, TPMT measurement during azathioprine therapy may be a more clinically relevant parameter than TPMT activity prior to the commencement of azathioprine in the assessment of efficacy.

The momentum to investigate the implications of TPMT activity in inflammatory bowel disease patients has steadily grown. In particular, it is recognized that patients with low TPMT activity are more susceptible to the development of bone marrow suppression side-effects.^{2, 3, 9} The impact of TPMT activity on the clinical course of inflammatory bowel disease patients treated with low-dose azathioprine has not been studied to date.

In this study, we aimed to retrospectively evaluate the clinical course of inflammatory bowel disease patients currently taking azathioprine and relate this to the respective TPMT activity.

MATERIALS AND METHODS

Patients

We recruited a cohort of 113 inflammatory bowel disease patients (52 ulcerative colitis, 61 Crohn's disease; 57 female, 56 male; median age, 45 years; range, 18–71 years) who were taking azathioprine, had discontinued azathioprine because of side-effects or who had never taken azathioprine. We compared these patients with a group of 17 healthy controls (eight female, nine male; median age, 39 years; range, 26–62 years). The median duration of follow-up was 4.7 years (range, 0.6–9.9 years). TPMT activity was determined from blood samples by a radiochemical assay, as detailed below. Relapse rates per year of follow-up (number of relapses divided by number of years of treatment) and time to first relapse were analysed and compared with the respective TPMT activity and the current azathioprine dose per kilogram body weight. The TPMT activity was classified as low if it was below 20 nmol/mL red blood cells/h. All cases were examined by one investigator (SC).

Other information collected included the lowest neutrophil count ever recorded during therapy, concomitant drug therapy, anatomical disease distribution, azathioprine dose and the patient's body weight at induction of therapy. Relapse was defined by necessity for surgery, endoscopic criteria or clinical recurrence (as assessed by an experienced clinician based on clinical features and inflammatory parameters — erythrocyte sedimentation rate, C-reactive protein concentration, white cell count and platelet count). Surgery for purely mechanical obstruction, without abnormal inflammatory parameters, was not considered to be a relapse of active disease. Endoscopic findings complemented clinical features and blood parameters, but were not used solely to determine relapse.

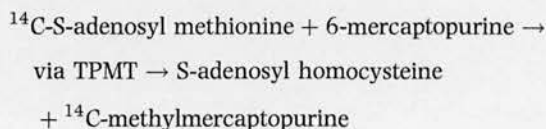
Blood samples

Blood was collected by venepuncture into 2.7 mL ethylenediaminetetra-acetic acid vacutainer tubes. Red blood cells were washed twice with pH 7.4 phosphate buffer solution and then centrifuged at 2000 *g* for 10 min. The haematocrit of a suspension of 0.5 mL red blood cells in 0.5 mL phosphate buffer solution was then measured. Finally, the suspension was haemolysed

with 2.8 mL of ice cold distilled water and 0.7 mL aliquots were pipetted into 1.5 mL Eppendorf tubes ready for the assay.

TPMT assay

The TPMT activity was measured in haemolysed red blood cells utilizing the following reaction:



Haemolysed red cells were spun at 11 600 *g* for 10 min in a microfuge centrifuge at 4 °C. For each sample, 100 μL red cell lysate was then added to 15 mL plastic tubes for blank and test estimation (both in duplicate). Twenty-five microlitres of pH 7.5 potassium phosphate buffer was added to each tube; this was followed by 5 μL 6-mercaptapurine for test estimation and 5 μL dimethylsulphoxide for blank estimation. The reaction was started by adding 25 μL of reagent mixture (containing $^{14}\text{C-S-adenosyl methionine}$, S-adenosyl methionine and dithiothreitol solution) and incubating at 37 °C in a shaking water bath for 1 h. The reaction was stopped by the addition of 500 μL of 0.5 M borate buffer, pH 10.0. The methylmercaptapurine was extracted into an isoamyl alcohol/toluene mixture and centrifuged at 1600 *g* for 10 min at room temperature; 1.5 mL of the organic phase was then added to 10 mL scintillant (OpticSafe, Pharmacia) and the radioactivity was counted for each of the vials for 10 min in a β -scintillation counter. The activity of TPMT in the red blood cell samples was calculated from the concentration of $^{14}\text{C-methylmercaptapurine}$ in the test sample (corrected for the blank sample), and the activity was expressed as nmol/mL red blood cells/h.

Statistical analysis

Relapse rates per year of follow-up were compared with the patients' TPMT activities by non-parametric Mann-Whitney test, and *P* values of ≤ 0.05 were regarded as significant. Time to first relapse was also compared by Kaplan-Meier curves using log rank analysis and chi-squared testing. Comparisons between multiple groups were performed using analysis of variance (ANOVA); $P \leq 0.05$ was considered to be significant. All statistical

analyses were performed using Minitab (release 13.20) statistical software.

RESULTS

One hundred and thirty blood samples were analysed from 113 inflammatory bowel disease patients and 17 healthy controls. The inflammatory bowel disease patients were categorized as 'azathioprine intolerant', 'azathioprine maintenance' or 'azathioprine naïve' (see Table 1). The 'intolerant' group were patients who had previously received azathioprine, but had subsequently discontinued treatment because of side-effects (pancreatitis, bone marrow suppression, abdominal pain, nausea, dermatological manifestations). The 'maintenance' group were patients who were currently taking and were stable on azathioprine therapy. The 'naïve' group were inflammatory bowel disease patients who had never received azathioprine treatment. Figure 2 illustrates the TPMT activity in these groups; the analysis comparing the control group against the other three groups was not significant by Mann-Whitney test.

The 'intolerant' category was then further analysed. Side-effects were individually grouped as neutropenia (neutrophil count, $< 2.5 \times 10^6$; mean TPMT activity, 19 nmol/mL red blood cells/h), pancreatitis (as defined by clinical symptoms and a raised amylase; mean TPMT activity, 24 nmol/mL red blood cells/h), hepatitis (raised alanine transaminase twice above baseline measurement; mean TPMT activity, 28 nmol/mL red blood cells/h), dermatological (skin rashes that resolved once azathioprine was stopped; mean TPMT activity, 27 nmol/mL red blood cells/h) and others (a variety of side-effects including non-specific abdominal pain, nausea, vomiting, taste disturbance and headaches; mean TPMT activity, 27 nmol/mL red blood cells/h). This is shown in Figure 3. The mean TPMT activity in the neutropenic patient group was significantly lower than that of patients who had other side-effects (ANOVA, $P < 0.05$).

To investigate the impact of TPMT activity on relapse rates in inflammatory bowel disease patients, we examined two groups. Both groups had taken azathioprine at a dose of less than 2 mg/kg for a minimum of 1 year. The groups were subdivided by their respective relapse rates as either 'non-relapsers' ($n = 20$) or 'relapsers' ($n = 14$) (i.e. more than zero relapse per year of follow-up). These groups were well matched for azathioprine dose (1.5 mg/kg vs. 1.45 mg/kg), duration

Table 1. Patient category characteristics

	n	M/F	Median age (range) (years)	Median AZA dose (range) (mg/kg)	Median duration AZA (range) (years)	Median TPMT activity (range) (nmol/mL RBC/h)	Concurrent 5-ASA	CD	UC
Controls	17	8/9	39 (26–62)	N/a	N/a	29 (20–35)	Nil	N/a	N/a
AZA intolerant	24	11/13	39 (21–66)	1.4 (0.6–2.3)	0.9 (0.1–4.6)	25 (14–41)	13	13 TI, 3 IC, 5 C, 5	11 P, 5 L, 4 D, 2
AZA maintenance	63	33/40	44 (18–69)	1.75 (1.4–2.4)	3.0 (0.6–9.9)	24 (9–35)	35	33 TI, 4 IC, 11 C, 18	30 P, 10 L, 11 D, 9
AZA naïve	26	13/13	48 (21–71)	N/a	N/a	24 (14–40)	16	15 TI, 6 IC, 4 C, 5	11 P, 2 L, 4 D, 5

5-ASA, 5-aminosalicylate; AZA, azathioprine; M/F, male/female; TPMT, thiopurine methyltransferase; CD, Crohn's disease; UC, ulcerative colitis; TI, terminal ileum; IC, ileocolonic; C, colonic; P, pan; L, left-sided; D, distal; N/a, not applicable; RBC, red blood cells.

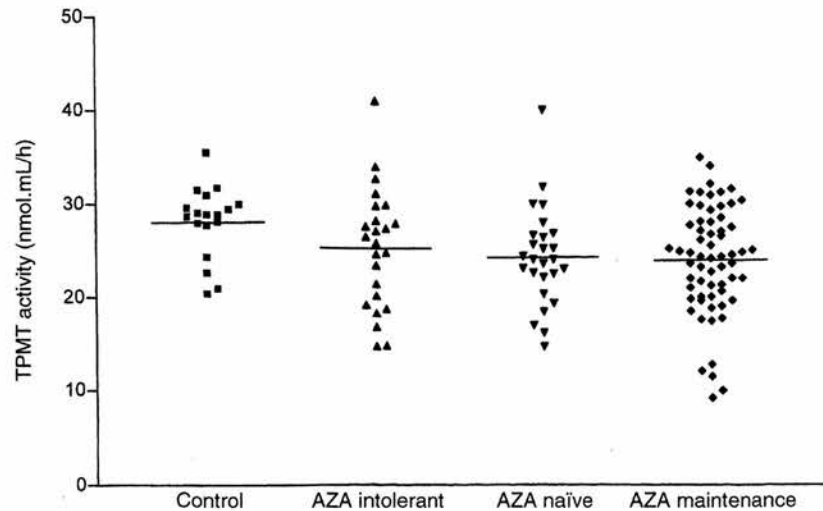
of follow-up (3.0 years vs. 2.6 years) and age (36 years vs. 35 years). The mean TPMT activity within the 'non-relapser' group was 19.8 nmol/mL red blood cells/h vs. 27.6 nmol/mL red blood cells/h for the 'relapser' group. This was statistically significant (Mann–Whitney test, $P < 0.005$). These results are summarized in Table 2. To further analyse this, a Kaplan–Meier survival curve was constructed based on the time to first relapse in the low-dose azathioprine-treated patients (< 2.0 mg/kg body weight; $n = 34$; 19 Crohn's disease, 15 ulcerative colitis) for TPMT activities of < 20 nmol/mL red blood cells/h and > 20 nmol/mL red blood cells/h (Figure 4.). Log rank analysis was statistically significant ($\chi^2 = 4.0$, $P < 0.05$). Further survival curves were constructed for Crohn's disease only and ulcerative colitis only, and log rank analysis was significant in both groups (for Crohn's disease: $\chi^2 = 3.2$, $P = 0.04$; for ulcerative colitis: $\chi^2 = 2.9$, $P = 0.05$).

Finally, the 'maintenance' group was divided into low TPMT activity (using the lower limit of our control group: < 20 nmol/mL red blood cells/h) or normal TPMT activity (> 20 nmol/mL red blood cells/h), and further survival curves were constructed based on time to first relapse for all inflammatory bowel disease patients. There were a total of 63 patients taking azathioprine (33 female, 30 male; 33 Crohn's disease, 30 ulcerative colitis; median age, 44 years). The mean follow-up in this group was 3.0 years, with a median azathioprine dose of 1.75 mg/kg (range, 1.4–2.4 mg/kg). This is shown in Figure 5. Log rank analysis did not show statistical significance ($\chi^2 = 0.2$, $P = \text{N.S.}$) despite a trend of a shorter time to relapse in patients with a TPMT activity > 20 nmol/mL red blood cells/h. Further analyses were performed on Crohn's disease only and ulcerative colitis only. Log rank analysis did not show statistical significance in either group (Crohn's disease: $\chi^2 = 0.22$, $P = \text{N.S.}$; ulcerative colitis: $\chi^2 = 0.06$, $P = \text{N.S.}$).

DISCUSSION

Low TPMT activity appeared to be predictive of a favourable response in patients treated with low doses of azathioprine (< 2 mg/kg). None of the patients in this cohort had extremely low TPMT activity (< 5 nmol/mL red blood cells/h), suggesting that none were homozygote recessive for the TPMT gene.⁷ As shown by other studies, there is an inverse relationship between red cell TPMT activity and 6-thioguanine levels in acute

Figure 2. Thiopurine methyltransferase (TPMT) activity plotted against subgroups of inflammatory bowel disease and controls (azathioprine (AZA) intolerant, $n = 24$; AZA maintenance, $n = 63$; AZA naïve, $n = 26$; controls, $n = 17$). Horizontal bars represent mean TPMT level of activity. None of the groups were statistically different from the control group.



lymphoblastic leukaemia, corresponding to a reduced risk of relapse but a higher probability of neutropenia.^{4, 5} This relation with neutropenia was also confirmed in this study. The TPMT activity correlated well with the lowest neutrophil count within the first 4 months of azathioprine therapy (Figure 6) in this group ($r = 0.41$, $P = 0.0001$). It should be noted that patients who became neutropenic within the first 4 months maintained this degree of neutropenia throughout azathioprine therapy. In addition, neutropenia has previously been shown to be of no advantage in keeping inflammatory bowel disease patients maintained in remission.¹⁰ This is further confirmed in this study by the lack of any difference in time to first relapse in all inflammatory bowel disease patients on azathioprine with low (< 20 nmol/mL red blood cells/h) or normal

(> 20 nmol/mL red blood cells/h) TPMT activities (Figure 5). Over the past two decades, it has become clear that, while azathioprine remains an essential second-line agent in the treatment of steroid-resistant or steroid-refractory inflammatory bowel disease, its relatively narrow therapeutic index means that patients may not fully benefit from a 'standard dose' of azathioprine.¹¹

TPMT activity is determined by a polymorphic gene in a co-dominant fashion, leading to a trimodal distribution of phenotypes,¹² and there is also evidence of ethnic variation in TPMT activity.^{13–15} Genotype $TPMT^L/TPMT^L$ gives rise to low TPMT activity and accounts for only 1% of the Western population.^{11, 14} These patients are particularly susceptible to serious bone marrow suppression side-effects.⁹

Figure 3. Thiopurine methyltransferase (TPMT) activity in different side-effect categories (neutropenia, $n = 8$; pancreatitis, $n = 4$; hepatitis, $n = 5$; dermatological, $n = 2$; other, $n = 7$). The horizontal bars represent the mean TPMT activity. TPMT values in the neutropenic group were significantly lower than those in the other side-effect groups (ANOVA, $P < 0.05$).

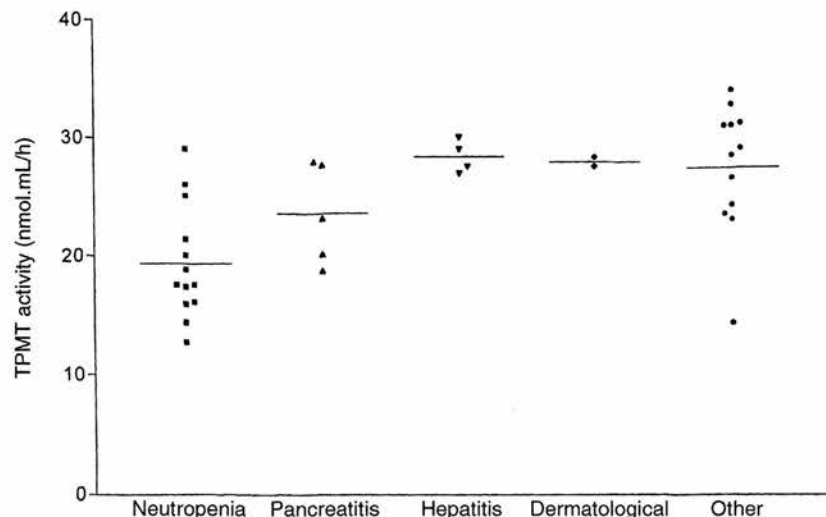


Table 2. Low-dose azathioprine patient characteristics

	<i>n</i>	F/M	Median age (range) (years)	Median AZA dose (range) (mg/kg)	Median AZA duration (range) (years)	Concurrent 5-ASA	Median relapse rate per year follow-up	CD	UC	Mean TPMT activity (nmol/mL RBC/h)	<i>P</i> *
Low-dose non- relapsers	20	12/8	36 (21–72)	1.50 (1–1.8)	3.0 (1.1–11.8)	11	0	TI, 3 IC, 3 C, 6	P, 3 L, 3 D, 2	19.8	< 0.0005
Low-dose relapsers	14	7/7	35 (24–52)	1.45 (1.2–1.8)	2.6 (1.8–2.9)	6	0.5	TI, 2 IC, 3 C, 2	P, 2 L, 2 D, 3	27.6	

5-ASA, 5-aminosalicylate; AZA, azathioprine; F/M, female/male; TPMT, thiopurine methyltransferase; CD, Crohn's disease; UC, ulcerative colitis; TI, terminal ileum; IC, ileocolonic; C, colonic; P, pan; L, left-sided; D, distal; RBC, red blood cells.
*Mann-Whitney test.

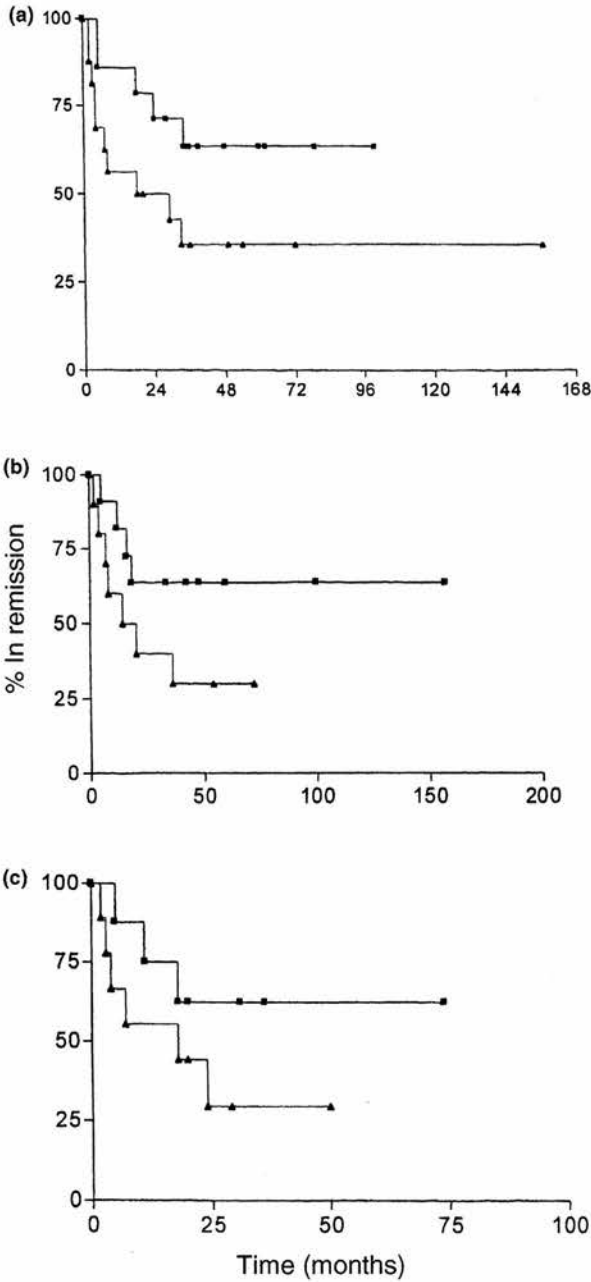


Figure 4. Survival curve. Time to first relapse in low-dose azathioprine (AZA) patients according to their thiopurine methyltransferase (TPMT) activity: (a) all inflammatory bowel disease (IBD) patients ($n = 34$, $\chi^2 = 4.0$, $P < 0.05$); (b) all Crohn's disease (CD) patients ($n = 19$, $\chi^2 = 3.2$, $P < 0.04$); (c) all ulcerative colitis (UC) patients ($n = 15$, $\chi^2 = 2.9$, $P < 0.05$). (■), < 20; (▲), > 20 nmol/mL red blood cells/h.

Although our cohort did not contain any patients with this phenotype, it may be possible to treat these homozygote recessive patients safely with azathioprine,

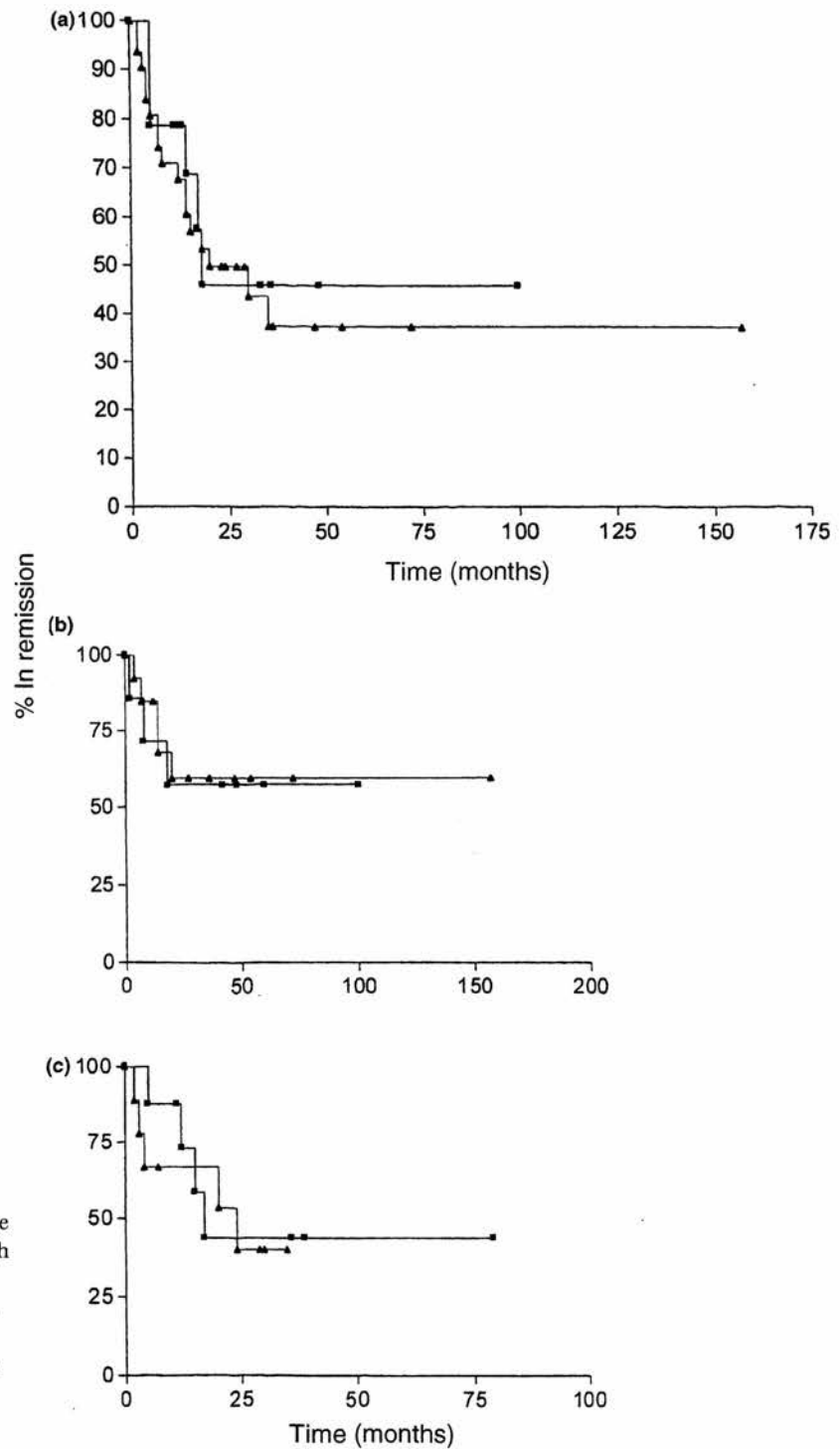


Figure 5. Survival curve comparison for all azathioprine (AZA) patients with low thiopurine methyltransferase (TPMT) activity vs. those with normal TPMT activity: (a) all inflammatory bowel disease (IBD) patients ($n = 63$, $\chi^2 = 0.2$, $P = \text{N.S.}$); (b) all Crohn's disease (CD) patients ($n = 33$, $\chi^2 = 0.22$, $P = \text{N.S.}$); (c) all ulcerative colitis (UC) patients ($n = 30$, $\chi^2 = 0.06$, $P = \text{N.S.}$). (■), < 20 ; (▲), > 20 nmol/mL red blood cells/h.

but at very low doses (< 0.5 mg/kg). Conversely, genotype $\text{TPMT}^{\text{H}}/\text{TPMT}^{\text{H}}$ (approximately 9% of the Western population^{11, 14}) leads to high TPMT activity, and hence these patients may benefit from higher doses

(> 2 mg/kg) of azathioprine that are not currently routinely prescribed in the UK. The majority of the population (90%) are heterozygotes $\text{TPMT}^{\text{H}}/\text{TPMT}^{\text{L}}$ giving a wide spectrum of TPMT activity.^{11, 14} It is this

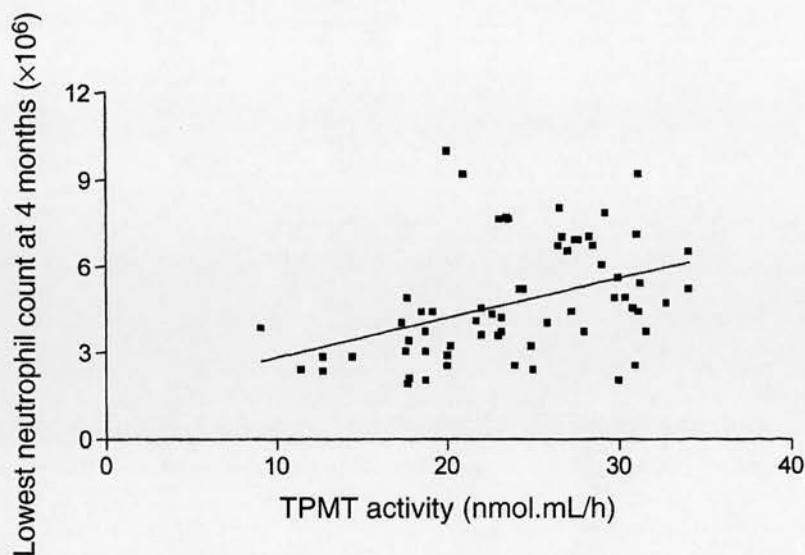


Figure 6. Plot of thiopurine methyltransferase (TPMT) activity against lowest neutrophil count per litre within first 4 months of treatment. $r = 0.41$, $P < 0.0001$.

majority of patients who would also further benefit by dose titration according to their TPMT activity, as shown by this study. There have also been concerns that low levels of TPMT activity may lead to secondary carcinogenic effects on bone marrow stem cells in leukaemic patients receiving chemotherapy protocols including 6-mercaptopurine.¹⁶ This clearly needs to be borne in mind, especially when contemplating long-term azathioprine therapy. However, it remains unknown whether 'dose adjustment' for lower TPMT activity may have the benefit of reducing such complications in the long term.

The principal finding of this study is that, if a low-dose regimen of azathioprine, i.e. < 2 mg/kg body weight is used, a knowledge of the TPMT phenotype is essential. If a low dose of azathioprine is used in inflammatory bowel disease patients with a high TPMT phenotype, the number of relapses suffered is likely to be high and the maintenance of remission imperfect. The higher doses of azathioprine generally recommended and used in the USA probably saturate this phenotypic variation in TPMT activity, resulting in higher overall remission rates. Although speculative, it is possible that a higher dose of azathioprine is only necessary in patients with higher TPMT activity. Our study results also provide an explanation for the commonly observed phenomenon of prolonged remissions on a low dose of azathioprine in a proportion of UK inflammatory bowel disease patients. In future, it may be possible to take into account the phenotypic variation of TPMT when deciding on the

exact dosage of azathioprine to be used in an individual patient.

This study used data from a large database of inflammatory bowel disease patients who were well matched for disease type and distribution, and there were comparable numbers of patients taking 5-aminosalicylate compounds to those who were not (Table 2). However, this is a retrospective analysis of patients and therefore subject to bias. In addition, patients' TPMT activities were measured while they were on concurrent medication, which may have induced or inhibited red cell TPMT activity. However, this was addressed by the fact that groups were well matched with regard to their concomitant medication type. The induction of TPMT activity by azathioprine remains a controversial issue. Studies reporting significant rises in TPMT activity after azathioprine have predominantly been in renal transplant patients^{7, 8, 17} and leukaemia patients.^{12, 18} These patients have been started on other concomitant medications that may also be responsible for TPMT induction in their own right, an observation reported previously with other commonly prescribed medications.² Furthermore, it has recently been shown that uraemia in renal transplant is a significant inducer of TPMT activity, making the role of azathioprine in the induction of TPMT less clear.⁷ TPMT induction is very variable and pre-treatment TPMT does not predict TPMT activity on azathioprine therapy. Only one study to date has addressed the issue of TPMT activity induction in healthy controls following the administra-

tion of 6-mercaptopurine, and reported no induction of TPMT activity.⁶ Finally, it is worth noting that, as some workers have reported somewhat erratic levels of TPMT activity induction following the commencement of azathioprine,¹⁹ it could be argued that a single TPMT activity measurement while stable on azathioprine therapy is more clinically relevant. To date, there have been no studies addressing these issues in inflammatory bowel disease patients. In our cohort, the distribution of TPMT activity was not different in those taking azathioprine compared with those currently not taking azathioprine. Further analyses have also been performed on patients taking 5-aminosalicylate medications, in view of recent concerns over *in vitro* 5-aminosalicylate enzyme inhibition.^{20–22} The mean TPMT activities in inflammatory bowel disease patients taking 5-aminosalicylate vs. those who were not were not statistically significantly different (data not shown), and this confirms the *in vivo* results published recently.²³

This is the first report addressing the issue of TPMT activity and disease relapse in inflammatory bowel disease patients to the authors' knowledge, opening up a further avenue in enabling the physician to individualize effective azathioprine therapy in these patients. We have shown that, in patients on a low dose of azathioprine (< 2 mg/kg), the mean TPMT activity was significantly lower in those in remission compared with those who relapsed during the follow-up period. The TPMT activity was significantly lower in patients who discontinued azathioprine due to neutropenia compared with those who discontinued azathioprine due to other side-effects.

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Azathioprine in Inflammatory Bowel Disease — Are We Using it Appropriately?

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Azathioprine (AZA) therapy is an invaluable second-line immunosuppressant, used in the treatment of IBD. Optimal use of AZA and 6-mercaptopurine continues to be refined with our growing experience of its use. However, questions remain unanswered regarding the use of these immunosuppressants during pregnancy, the place of thiopurine methyltransferase enzyme-activity monitoring to reduce potential side effects, concurrent use of 5-aminosalicylic acid drugs in the role of maintenance therapy, and the potential future role of red-cell metabolite monitoring in optimizing AZA drug therapy. This article reviews current publications to help shed more light on these issues.

Azathioprine (AZA) and 6-mercaptopurine (6-MP) are purine analogs that interfere with nucleic acid metabolism and cell proliferation, and thus have immunosuppressive properties. AZA, the pro-drug form, was produced in 1957, and was first used predominantly for preventing rejection in renal allografts [1]. Since then, AZA, and to a lesser extent 6-MP, have been regarded as the treatment choice for IBD patients who are steroid refractory or dependent.

Despite several conflicting reports, large meta-analyses have largely confirmed both the safety and efficacy of the drug in maintaining remission, and answered several questions concerning dosage, and time to onset of action. Despite this, the use of AZA remains small, with a Swedish study revealing that AZA accounts for as little as 2.2% of the annual drug prescriptions for Crohn's disease patients, and 0.5% for ulcerative colitis patients [2]. In addition, there is considerable heterogeneity between countries in the prescribing patterns of immunosuppressants [3]. For example, AZA is prescribed more often in Europe than North America, but the reverse is true for 6-MP.

There still remain many unanswered questions regarding the optimal use of AZA in the clinical setting, and the purpose of this article is to cover these topics with evidence from currently published work.

Mechanism of action

AZA is the prodrug of 6-MP. AZA is rapidly converted to 6-MP by a non-enzymatic pathway that is dependent on glutathione and other sulfhydryl-containing proteins and, subsequently, 6-MP is left available to the circulation. AZA is about 55% 6-MP by weight and, since over 75% of AZA is converted to 6-MP, 1 mg of AZA is equivalent to 0.5 mg of 6-MP [4].

Bioavailability of these drugs is low (<20%) due to the extensive catabolism by xanthine oxidase (XO) found in enterocytes and hepatocytes (but not hemopoietic tissue) [5]. 6-MP can be taken down two remaining pathways (Fig. 1) [6,7]. It can be:

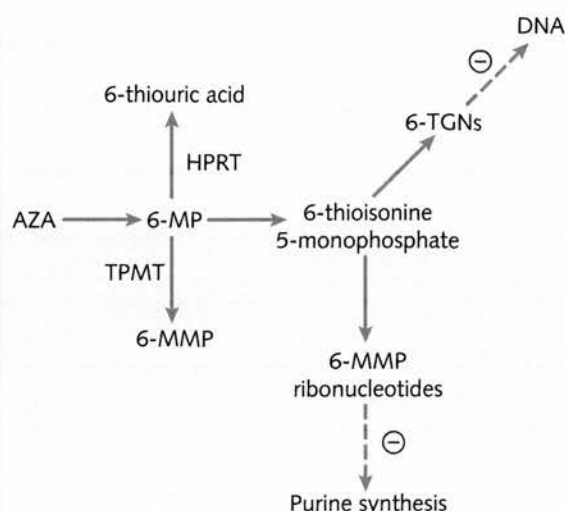
- converted by thiopurine methyltransferase (TPMT) to an inactive form, 6-methylmercaptopurine (6-MMP)
- anabolized by hypoxanthine guanine phosphoribosyl transferase (HPRT) to its active metabolites, 6-thioguanine nucleotides (6-TGs) and 6-MMP ribonucleotides (6-MMPRs).

Thus, 6-MP's mode of action rests on the incorporation of these thiopurine nucleotide metabolites into cellular nucleic acids, thereby inhibiting *de novo* purine synthesis [8]. These enzymatic pathways are distinct and competitive.

Toxicity

The main limiting factor in AZA use is the occurrence of side effects. Between 10–15% of patients who are

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Figure 1. AZA metabolism.

AZA: azathioprine; HPRT: hypoxanthine phosphoribosyltransferase; 6-MP: 6-mercaptopurine; 6-MMP: 6-methylmercaptopurine; 6-TGN: 6-thioguanine nucleotide; TPMT: thiopurine methyltransferase; ⊖: inhibition of synthesis.

Table 1. Azathioprine- and 6-mercaptopurine-related toxic reactions, and their percentage occurrence in treated patients.

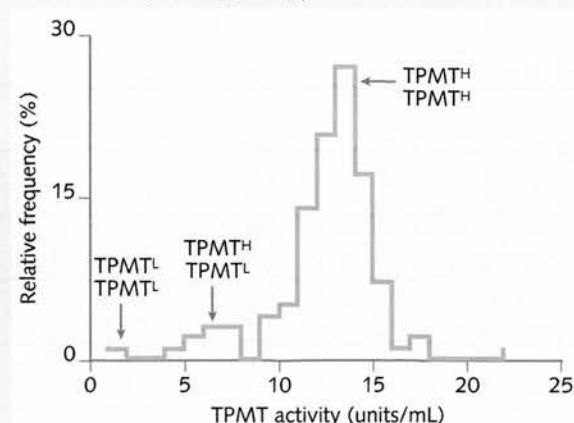
Toxic reaction	% occurrence
Infection	40
Bone marrow suppression	15
Hepatitis (mild derangement of liver function tests, intrahepatic cholestasis, irreversible liver injury)	3
Malignancy (non-Hodgkin's lymphoma, skin cancer, hepatoma, colorectal cancer)	10
Pancreatitis	20
Other allergic type reactions (nausea, abdominal pain, fever, skin rashes)	12

started on AZA will have some form of toxic reaction [9]. Broadly speaking, there are two categories of toxic reactions, namely allergic type (e.g. pancreatitis, fever, rash, non-specific abdominal pain, or nausea) and non-allergic type (e.g. leukopenia, thrombocytopenia, infections, malignancies, and some cases of hepatitis) (Table 1). Non-allergic-type reactions usually occur after months or years and appear to be dose dependent, while allergic-type reactions occur much earlier, usually within weeks. Table 1 summarizes the occurrence of toxic reactions to AZA and 6-MP.

Pharmacogenetics

TPMT

TPMT is a cytosolic enzyme. TPMT levels are determined by a well-documented co-dominant genetic polymorphism in Caucasian and Afro-American

Figure 2. Trimodal frequency distribution of red cell TPMT activity with genotypes.

TPMT^H: high thiopurine methyltransferase; TPMT^L: low TPMT. TPMT^H/TPMT^H: homozygous normal TPMT. TPMT^H/TPMT^L: heterozygous intermediate TPMT. TPMT^L/TPMT^L: homozygous low TPMT.

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populations [10,11]. Approximately 90% of the population has normal expression of TPMT (TPMT^H/TPMT^H), 9% have intermediate activity due to heterozygosity at the TPMT locus (TPMT^H/TPMT^L), while 1% have a TPMT deficiency (TPMT^L/TPMT^L), which is inherited as an autosomal recessive trait [11]. In such patients, there is excessive shunting of by-products down other enzymatic pathways, leading to excesses in 6-TGs, which exert cytotoxic and immunosuppressive effects leading to bone-marrow toxicity. Other adverse side effects, such as pancreatitis and hepatitis, are thought to be idiosyncratic reactions, unrelated to TPMT activity [12].

There has been considerable interest in TPMT levels and the occurrence of AZA/6-MP toxicity. This was first investigated in children with acute lymphoblastic leukemia, treated with AZA, where it was recognized that TPMT levels corresponded to a reduced risk of relapse, but a higher risk of neutropenia [13,14]. In addition, TPMT genotyping has been shown to correlate well with TPMT enzymatic activity [15] and follows a trimodal distribution (Fig. 2). TPMT activity can be measured in red blood cells by a variety of methods, including reverse-phase high-performance liquid chromatography (HPLC) [16], and by radio-incorporation assay [17,18]. To date, there have been few studies looking at TPMT activity and its associated toxicity in IBD patients. TPMT genotyping (shown to reflect enzymatic activity) has been shown to correlate well with myelotoxicity, but otherwise does not predict hepatotoxicity or the occurrence of pancreatitis.

It should be remembered that while TPMT measurements (genotypic and activity) clearly appear to

have a useful role in identifying patients susceptible to myelotoxicity, other environmental factors can play a role in the development of drug toxicity such as parvovirus B19 infection [19]. This means that blood-count monitoring remains essential, since severe myelosuppression can occur despite normal TPMT levels.

HPRT

HPRT deficiency occurs in those patients with raised urate levels who are predisposed to gout. In its severest form, the deficiency presents in childhood, with associated neurologic defects, and is known as Lesch–Nyhan syndrome. The gene that codes for HPRT is cross-linked, and there are several described mutations along this gene that cause a variable degree of deficiency in HPRT levels. Such is its rarity, that deficiency of HPRT is probably of little clinical relevance with respect to AZA therapy in IBD patients. However, these patients would be resistant to the immunosuppressive effects of AZA therapy [20].

XO

It is well recognized that allopurinol (which inhibits XO) interacts with AZA, and can precipitate toxicity. XO catalyzes the conversion of 6-MP to thiouric acid (inactive), hence, genetically low levels of XO leave more 6-MP available for conversion into 6-TGs. XO deficiency is an autosomal recessive disorder, occurring in about 2% of the population, and is often discovered incidentally by the presence of hypouricemia [21]. Like TPMT deficiency, this condition is associated with hemotoxicity, but has received only modest interest when compared with TPMT-associated toxicity.

AZA — evidence-based medicine?

There has been a paucity of good, randomized, double-blinded studies into the use of AZA and 6-MP in IBD. In addition, trials in Crohn's disease patients have generally been better represented than similar trials in ulcerative colitis patients. However, there has been a more thorough analysis undertaken through meta-analysis, leading to more positive support for the efficacy of AZA and 6-MP in IBD patients. There have never been any direct comparative studies between AZA and 6-MP, but it is reasonably assumed that both drugs have similar effects, after taking into account the obvious dose differences mentioned earlier.

Crohn's disease

Active disease

There have been only four studies that have addressed treatment exclusively in active disease [22–25]. These studies were quite heterogeneous in respect to the outcomes (steroid-sparing effect, development of remission, etc.) and study design (crossover versus

non-crossover), but all compared placebo with AZA. Rhodes et al. found no difference in the response of patients to AZA compared with placebo, but the study was flawed by the short duration of therapy (7 weeks) [25]. All of the other studies found varying degrees of favorable response (odds ratio [OR] 1.0–10.45) [22–24,26–28], and also found a steroid-sparing effect. A meta-analysis of the results, by Pearson et al. [29], included multi-arm studies that looked at active disease and steroid-sparing effect. The estimated overall common odds ratio [OR] was 3.09 (95% CI 2.45–3.91) in favor of AZA or 6-MP therapy (Table 2).

Maintenance therapy

Two studies have looked exclusively at treatment with AZA in quiescent disease [30,31], several other studies have included maintenance therapy as part of a multi-arm study [26–28]. Again, all of these exhibited varying degrees of benefit (OR 1.20–4.48), and meta-analysis by Pearson et al. estimated a common OR of 2.27 (95% CI 1.76–2.93) in favor of AZA therapy (Table 3) [29].

Ulcerative colitis

Active disease

The use of both AZA and 6-MP in active colitis is limited due to the protracted time to maximal onset (approximately 3 months). There is no evidence that AZA plus steroids, versus steroids alone, increases remission rates, but there is evidence of a steroid-sparing effect in the AZA group within this time frame [32,33].

Maintenance therapy

AZA therapy has been studied more extensively than 6-MP in ulcerative colitis. There is good evidence that both AZA and 6-MP allow a steroid-dose reduction, a conclusion found in several studies to date (AZA dose 1.5–2.5 mg/kg) [32–36]. It should be noted that there is no clear evidence to show that AZA or 6-MP are of benefit in the chronic active ulcerative colitis patient who is steroid resistant. Jewell and Truelove studied such patients for 12 months and found no clear benefit [35], although uncontrolled data from others has shown beneficial effects [37]. Further evidence can be drawn from withdrawal studies, which have shown that AZA withdrawal during remission leads to increased relapse rates [38]. There are no controlled, randomized trials that have looked exclusively at 6-MP and maintenance therapy in ulcerative colitis.

Current issues

Optimal antimetabolite therapy is a minefield in current practice. This is partly due to poorly designed studies, but more importantly, a lack of specific studies

Table 2. Odds ratio of response in randomized controlled studies of azathioprine and 6-mercaptopurine for active Crohn's disease.

Study	Odds ratio (95% CI)
Rhodes et al. [25]	1.00 (no CI, no responders)
Klein et al. [23]	1.00 (0.22–4.54)
Candy (Part 1) et al. [26]	1.55 (0.52–4.59)
Summers et al. (National Cooperative Crohn's Disease Study [Part I, Phase 1]) [27]	1.57 (0.75–3.29)
Ewe et al. [22]	4.57 (1.36–15.27)
Present et al. [24]	10.45 (4.14–26.38)
Willoughby et al. (Group 1) [28]	23.17 (2.57–99.9)
Common odds ratio	3.09 (2.45–3.91)

CI: confidence interval.

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looking at unanswered issues. Since there has been more extensive research into AZA and other medical conditions, the conclusions drawn from this have been extrapolated for use in IBD therapy, but it should be noted that, equally, some conclusions may not apply to IBD patients.

Intravenous AZA use

In the light of the delayed onset of action of oral AZA, there has been growing interest in the use of intravenous AZA in the hope of decreasing the time of therapeutic onset, and increasing the therapeutic capability of AZA in acute ulcerative colitis. TPMT activity status is a prerequisite to using AZA intravenously, to stop potentially fatal side effects. The first published experience in IBD was by Sandborn et al. in 1995, where Crohn's disease patients were loaded with 1800 mg of AZA over 36 h [39]. The authors concluded that there was a reduced time-to-response in these patients. Subsequently, Casson et al. presented three patients with acute fulminant colitis (one with ulcerative colitis, one with indeterminate colitis, and one with Crohn's disease) who were treated with low-dose intravenous AZA (3 mg/kg/daily) for 5–7 days [40]. All three patients achieved remission on this treatment. There have been two more publications, the first, by Sandborn et al. in 1999, was a placebo-controlled trial of an intravenous loading dose of AZA (40 mg/kg/36 h) in 51 patients versus 45 placebo-treated 'control' patients — both patient groups having steroid-resistant Crohn's disease [41]. There was no decrease in time-to-response in the AZA-treated patients, despite achieving higher 6-TGN concentrations at 0.2 weeks and 1 week. Thereafter, 6-TG concentrations in both groups were the same, hinting that oral AZA therapy achieves similar 6-TG levels to intravenous AZA. Finally, Mahadevan et al.

Table 3. Odds ratio of response in randomized controlled studies of azathioprine for quiescent Crohn's disease.

Study	Odds ratio (95% CI)
National Cooperative Crohn's Disease Study, (Part II) [27]	1.20 (0.60–2.41)
National Cooperative Crohn's Disease Study, (Part I, Phase 2) [27]	1.73 (0.37–8.05)
O'Donoghue et al. [30]	2.95 (0.97–9.00)
Rosenberg et al. [31]	3.16 (0.57–17.62)
Willoughby et al. (Group 2) [28]	4.48 (0.41–49.43)
Candy et al. (Part 2) [26]	7.12 (2.11–23.99)
Common Odds Ratio	2.27 (1.76–2.93)

CI: confidence interval.

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looked at acute ulcerative colitis in nine patients [42]. Three groups, of three patients each, were treated with 20 mg/kg/36 h, 40 mg/kg/36 h, or 40 mg/kg as three separate 8-h infusions over 3 days. Forty-six percent of these patients avoided colectomy, and a response was seen within 4 weeks, with therapeutic 6-TG concentrations being achieved by 12 weeks. They concluded that intravenous AZA appeared to be safe and of clinical benefit in steroid refractory ulcerative colitis patients.

AZA dose

Current practice involves the use of a low dose of AZA/6-MP for the first 6–8 weeks, with subsequent dose adjustment to approximately 2 mg/kg (or even higher, if tolerated by the patient). There is evidence that doses of 1.5–2.5 mg/kg are effective in IBD; this has been found in both single studies and meta-analyses [32,33,36]. Doses <1.5 mg/kg probably have little clinical efficacy, and there is strong evidence that a dose of 1 mg/kg is ineffective in Crohn's disease. There are no studies that have directly compared different doses of either AZA or 6-MP to look at either relapse rates or the degree of steroid-sparing effect. In addition, there have been no trials looking at doses of >3 mg/kg.

Concurrent 5-aminosalicylic acid use

The concomitant use of 5-aminosalicylic acid (5-ASA) with AZA may have potential advantage in reducing the long-term colorectal malignancy risk associated with IBD. On the other hand, there is rising concern over the possible interaction of 5-ASA compounds, and their breakdown products, with TPMT [43]. *In vitro* studies have suggested that 4-ASA, 5-ASA, and sulphasalazine all inhibit TPMT activity to differing degrees [44–46]. There is only one study to date that has addressed the question as to whether concomitant 5-ASA therapy is required in patients maintained in remission with

AZA [47]. This retrospective study looked at 169 IBD patients maintained on AZA (with and without concurrent 5-ASA), with a median follow-up duration of 3.5 years. Relapse rates and side effect frequency in the two groups were also examined and no differences were found. Until there are further prospective studies addressing this issue, it would seem sensible to add in AZA/6-MP therapy with concurrent 5-ASA treatment, where indicated, and to be aware of a potential drug interaction. In addition, there is no current evidence that 5-ASA therapy should be stopped if a patient is stable on AZA/6-MP, or that the addition of 5-ASA is beneficial in patients established on AZA/6-MP.

Neutropenia

It is a matter of debate whether neutropenia is a prerequisite for optimal AZA therapy. In current clinical practice, AZA dose is not titrated against neutropenia, unless dangerous levels of neutropenia are seen.

There has been interest in the role of AZA- or 6-MP-induced leukopenia in accomplishing disease remission [48]. In addition, there have been anecdotal reports of prolonged remission in patients who have sustained pancytopenia during AZA therapy [49]. It has been proposed that the preferential suppression of neutrophils during leukopenia affects remission induction with AZA/6-MP. A large, retrospective study by Campbell and Ghosh [50] examined relapse rates per year of follow-up in a cohort of 173 IBD patients, as well as time-to-first-relapse in patients whose lowest neutrophil counts were classified as neutropenic ($<2.5 \times 10^9$) versus those with non-neutropenic counts ($>2.5 \times 10^9$). There were no differences in relapse rates between the two groups and, at present, this would suggest that neutrophil count should not be used as an endpoint to dose titration of AZA or 6-MP.

Mean corpuscular volume

It has long been recognized that AZA/6-MP causes blood-count abnormalities, such as raised leukopenia, thrombocytopenia, and raised mean corpuscular volume (MCV). The latter parameter is not a sign of toxicity. The mechanism by which MCV increases is thought to be related to interference of cell proliferation in blood precursors committed to erythropoiesis [51]. The rise in MCV following administration of AZA/6-MP is not necessarily inevitable, and the level that the MCV rises does not correlate with drug dosage [52].

MCV changes (δ MCV) have been studied extensively in renal transplant patients, but currently no studies have been published in IBD patients. More recently, δ MCV during therapy have been shown to be a reasonable indirect estimation of 6-TG levels. However, a prospective study should be undertaken

before this parameter is utilized in clinical practice [53]. Levels of 6-TG measured directly have been shown to correlate well with the actual bioavailability of oral AZA/6-MP, and, hence, with the ability to retain disease remission during therapy.

6-TG monitoring

There has been increasing interest in the use of monitoring 6-MP metabolite levels as a more accurate method of assuring drug response. Erythrocyte 6-TG levels represent an indirect measure of bone marrow uptake and hence the degree of immunosuppression. Red cell 6-TG levels can be measured by a modification of a reversed-phase HPLC method, initially used to measure TPMT levels [16]. 6-TG levels have been shown to exhibit a significant inverse correlation with the Harvey Bradshaw index (HBI) activity index in Crohn's disease [54]. Thus, the measurement of 6-TG levels is useful in assessing both drug compliance and responsiveness to treatment, while preventing under- and over-dosing.

Pregnancy and fertility issues

Clinicians are not under any obligation to report the outcome of the administration of drugs during pregnancy to a central monitoring authority. Therefore, data concerning both pregnancy and fertility in patients taking AZA is confined to the voluntary reports submitted for publication, and those cases reported to the pharmaceutical company. Initially, published literature focused on renal transplant patients, subsequently, smaller and less-detailed reports have been published on this, and other medical conditions, such as rheumatoid arthritis, systemic lupus erythematosus, autoimmune hepatitis, and IBD [55–58]. There is no doubt that AZA crosses the placenta and is expressed in the breast milk of mothers taking AZA. However, the concentrations in the placental tissue are small (representing up to 14% of the orally administered dose), and thus it appears unlikely that such metabolite concentrations will have a clinically significant effect on the fetus [59]. Cytogenetic analysis has detected chromosomal aberrations in the offspring of mothers with Crohn's disease, but long-term follow-up has not revealed further problems in these infants [28]. Immunologic abnormalities (e.g. lymphopenia, thymic hypoplasia, and bone marrow hypoplasia) have also been reported in the newborn offspring of these female patients [60,61], but all of these have subsequently resolved later in infancy. Unfortunately, there is a lack of reports of detailed immunologic assessments in these patients.

Overall, good control of the patient's IBD is much more important for pregnancy and the well-being of the fetus. It should be remembered that patients with

Figure 3. Key points.

- On current evidence, AZA is safe during pregnancy.
- TPMT activity monitoring is useful in reducing the occurrence of associated bone marrow toxicity.
- Concurrent use of 5-ASA drugs is probably unnecessary in patients maintained in remission on AZA.
- The degree of neutropenia associated with AZA therapy does not relate to efficacy of maintaining remission in IBD patients.
- Optimal dosing of AZA should be achieved and, in future, the measurement of 6-TG metabolites may help with individualizing the optimal dosage of AZA.

ASA: aminosalicylic acid; AZA: azathioprine; TG: thioguanine; TPMT: thiopurine methyltransferase.

Crohn's disease have higher rates of spontaneous abortion and stillbirth, and that controlling disease activity may reduce these risks [55].

Spermatogenesis has been studied in renal transplant patients and shown to be similar to healthy controls [62], other small reports in renal transplant patients revealed decreased sperm counts, but the counts remained within the normal range [63].

Neoplasia risk

It has been well recognized that the risk of various malignant disorders is higher in transplant recipients receiving immunosuppressive therapy than in the general population. In particular, prospective studies have shown an excess of non-Hodgkin's lymphoma (NHL), squamous cell carcinoma, and hepatoma [64–66]. In addition, rheumatoid arthritis patients receiving AZA have been shown to exhibit excessive frequencies of these malignancies beyond those associated with rheumatoid disease [67]. Probably the largest IBD study was by Connell et al. in 1994, who followed 755 IBD patients taking AZA (2 mg/kg), over a 29-year period [68]. The group concluded that, while colorectal carcinoma occurred in excess (something observed in IBD patients anyway), there were no cases of NHL. Another study by Present et al. looked at 396 IBD patients, treated with 6-MP for a mean period of 5.4 years. They described 12 cases of malignancy, one of which was NHL [9]. Finally, a large analysis of 782 IBD patients by Farrell et al. concluded that, although there was an increased risk of NHL, the overall risk was low [69]. Clearly, as long as patients are informed of the potential and seemingly small risks of AZA therapy, it seems sensible that AZA should be used considering the morbidity associated with other long-term medical therapies and risks from surgery.

Duration of therapy

There have now been several long-term studies looking at both AZA and 6-MP use in Crohn's disease and ulcerative colitis. Probably the most quoted paper is that

from Bouhnik et al. that first addressed this issue [70]. They looked at 157 patients over a 6-year period. Relapse rates were examined in patients on AZA/6-MP compared with those whose AZA/6-MP therapy was discontinued. They concluded that beyond 4 years of therapy, relapse rates were the same for both groups. It should be noted that at this time point, the two groups were small and, hence, may not have had the power to detect a significant difference in relapse rates. A smaller study involving 105 ulcerative colitis patients came to a different conclusion [37]. Duration of follow-up was approximately 5 years, but relapse in the withdrawal group was very small (n=15) making conclusions somewhat difficult to interpret. More recently, Kim et al. looked at 6-MP therapy in 120 Crohn's disease patients with a follow-up period of >8 years [71]. Again, study design was similar, but final conclusions found statistical significance between 6-MP and the withdrawal group. Their conclusions advocated the indefinite use of 6-MP therapy, once remission was achieved. However, until there is a placebo-controlled withdrawal trial, optimal duration of therapy is unlikely to be known.

Clearly, there appears to be good evidence for the use of AZA/6-MP therapy of up to 4–5 years and, if the drug is well tolerated, longer therapy may well be indicated.

AZA or 6-MP therapy?

Although the side-effect profiles of both AZA and 6-MP are very similar, there is evidence of differences in the bioavailabilities of these two drugs, and between those of branded AZA and generic AZA [72]. This has further implications with regard to optimizing drug treatment with 6-TG monitoring. In addition, there has been a small series of patients who have been re-challenged with 6-MP after being intolerant to AZA. In a group of 11 patients, 54% were able to tolerate 6-MP [6], hinting that 6-MP may have benefits over AZA for initial therapy [73]. More studies are required before any firm conclusions can be drawn.

Conclusion

In summary, antimetabolite therapy has proven itself an extremely valuable and efficacious tool that now forms an invaluable addition to the armament of the gastroenterologist in helping treat steroid refractory/dependent IBD. Although there may well be a great number of small and poorly designed studies, experience has given us an increased understanding, and has helped to develop ever more optimal immunosuppressive therapy for IBD patients (Fig. 3). Its long-term safety is now generally well accepted, and with appropriate monitoring and with more studies, further information will help us refine antimetabolite use for the future.

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Is neutropenia required for effective maintenance of remission during azathioprine therapy in inflammatory bowel disease?

Simon Campbell and Subrata Ghosh

Background Azathioprine is an effective treatment for maintaining remission in inflammatory bowel disease (IBD). It is a matter of debate as to whether neutropenia is required during azathioprine therapy to achieve more effective disease remission. We evaluated whether neutropenia during azathioprine therapy reduced relapse rates in IBD patients.

Patients and methods This retrospective study was based on a total of 173 IBD (96 Crohn's disease (CD), 77 ulcerative colitis (UC)) patients who were stable on azathioprine for a minimum of 6 months. Median duration of follow-up was 4.0 years (range 0.6–21 years). The lowest neutrophil counts during treatment for these patients were recorded. Relapse rates per year of follow-up were compared in non-neutropenic patients (neutrophil count $> 2.5 \times 10^9$, $n = 129$) and neutropenic patients (neutrophil count $\leq 2.5 \times 10^9$, $n = 44$) groups, and survival curves for cumulative remission rates compared by log-rank test.

Results Mean relapse rate per year of follow-up for the non-neutropenic group was 0.19/year (SD = 0.37/year) compared with the neutropenic group 0.28/year (SD = 0.43/year) ($P = \text{NS}$). Analysis was performed on UC

and CD subgroups, and relapse rates were not significantly different. The cumulative remission per cent determined by Kaplan–Meier survival analysis showed no difference between non-neutropenic and neutropenic groups by log-rank analysis, for UC and CD as well as for all IBD patients.

Conclusion Neutropenia $\leq 2.5 \times 10^9$ while on azathioprine does not reduce the relapse rates of IBD patients who were established on azathioprine therapy compared with neutrophil counts $> 2.5 \times 10^9$. *Eur J Gastroenterol Hepatol* 13:1073–1076 © 2001 Lippincott Williams & Wilkins

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Keywords: azathioprine, Crohn's disease, inflammatory bowel disease, neutropenia, ulcerative colitis

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Introduction

Azathioprine use in inflammatory bowel disease (IBD) is now widely accepted as an invaluable second-line immunosuppressive agent in the treatment of steroid-refractory or steroid-dependent disease [1,2]. The optimal treatment regimen for azathioprine use continues to be defined as our experience with its use in IBD grows.

Azathioprine is usually introduced at a low dose and titrated up to an arbitrary dose of approximately 2 mg/kg, although higher doses are generally used in the USA. The mean time to onset of effect of azathioprine is about 3 months, and it is generally accepted that a dose of 2–2.5 mg/kg is efficacious. Azathioprine is broken down to 6-mercaptopurine and then to active 6-thioguanine by the enzyme thiopurine methyltransferase (TPMT). TPMT is genetically determined, and is inherited in a co-dominant fashion [3]. Genetically low levels of TPMT have shown to correlate well with the

occurrence of some azathioprine-induced side effects, in particular the development of neutropenia [4].

Both macrocytosis and lymphopenia are well-recognized haematological effects of azathioprine therapy, and their relevance to achieving or maintaining remission remains uncertain [5,6]. The dangers of leucopenia and, perhaps more importantly, neutropenia have long been recognized, necessitating regular blood count monitoring of patients taking azathioprine.

More recently, there has been interest in the potential therapeutic value of 'safe' levels of neutropenia and leucopenia in achieving and maintaining remission [5,7]. Anecdotal reports have suggested that these may be now desirable end points to azathioprine therapy [8]. However, this remains unproven, and in current clinical practice azathioprine dose is not titrated against neutropenia, unless dangerous levels of neutropenia are seen.

Patients and methods

A database was constructed containing information on IBD patients maintained on azathioprine ($n = 203$; ulcerative colitis (UC) = 94; Crohn's disease (CD) = 109; median age 39 years, range 15–82 years; 88 male, 115 female). For the purposes of this study, patients were excluded if they were not maintained on azathioprine for a minimum of 6 months, or if treatment was stopped due to non-haematological side effects, or if the disease remained active despite azathioprine therapy. This left a total of 173 IBD patients (UC = 77, CD = 96; median age 39 years, range 16–81 years; 76 male, 97 female) who had been maintained in remission on azathioprine. Median duration of follow-up was 4.0 years (range 0.6–21 years). Other information that was collected included the lowest neutrophil count recorded during therapy, the lowest neutrophil count within the first 4 months of therapy, anatomical disease distribution, azathioprine dose, patient's weight at induction of therapy, and smoking status. Relapse was defined as necessity for surgery, or clinical recurrence (as assessed by an experienced clinician based on clinical features of diarrhoea, abdominal pain, general wellbeing, weight loss and inflammatory parameters – haemoglobin, erythrocyte sedimentation rate (ESR), C-Reactive Protein (CRP), white cell count and platelets). Requirement for surgery for purely mechanical obstruction, without abnormal inflammatory parameters, was not considered a relapse of active disease. Endoscopic findings complemented clinical features and blood parameters, but were not used solely to determine relapse. Relapse rates per year of follow-up were compared with the lowest degree of neutropenia achieved during azathioprine therapy. A neutrophil count of $\leq 2.5 \times 10^9$ was used to define neutropenic patients. All cases were examined by one investigator (SC).

There were 44 patients who had been neutropenic (mean neutrophil count 1.9×10^9 , SD 0.34) while taking azathioprine, with 129 patients not achieving any neutropenia (mean neutrophil count 3.7×10^9 , SD 0.9). Doses of azathioprine were similar in both groups of patients (neutropenic: 1.8 mg/kg, SD 0.41; UC 1.9 mg/kg, SD 0.42; CD 1.75 mg/kg, SD 0.43; non-

neutropenic: 1.9 mg/kg, SD 0.47; UC 1.85 mg/kg, SD 0.48; CD 1.95 mg/kg, SD 0.45). Also comparable were mean duration of follow-up, duration of therapy, disease anatomy, concurrent use of steroids, and use of 5-aminosalicylic acid (5-ASA) drugs. These results are summarized in Table 1.

Statistical analysis was by non-parametric Mann–Whitney test (MWT) for comparing the two groups. Relapse rates were calculated using Kaplan–Meier survival curves and compared using log-rank test analysis.

Results

A total of 173 IBD patients were stable on azathioprine for a minimum of 6 months. Median duration of therapy was 4.0 years (range 0.6–21 years). Forty-four patients (25%) became neutropenic (lowest neutrophil count achieved during therapy $\leq 2.5 \times 10^9$) while taking azathioprine, of which four patients stopped treatment due to severe life-threatening neutropenia (2 CD, 2 UC), and were included in the final analysis. The two CD patients suffered from severe sepsis. One was from a urinary tract infection (lowest neutrophil count 0.5×10^9), which resolved with intravenous antibiotics. The other patient developed neutropenic sepsis necessitating the use of granulocyte colony stimulating factor (G-CSF, Filgrastim, Roche, Welwyn Garden City, UK) (lowest neutrophil count 0.2×10^9), intensive care admission for 10 days and haemodialysis, but made a full recovery. One of the UC patients developed severe pancytopenia and subsequent adult respiratory distress syndrome (ARDS; lowest neutrophil count 0.8×10^9), necessitating intensive care treatment for 5 days, but made a full recovery. The other UC patient developed no complications (lowest neutrophil count 0.8×10^9), and the blood count returned to normal within 1 week of stopping azathioprine.

For the UC patients (M = 22, F = 56), the neutropenia group (mean azathioprine dose 1.90 mg/kg, SD 0.42; M = 11, F = 17) had a mean relapse rate of 0.30/year follow-up (SD = 0.48) compared with 0.18/year follow-up for the non-neutropenic group (mean azathioprine dose 1.85 mg/kg, SD 0.48; M = 11, F = 39; $P = 0.63$

Table 1 Patient characteristics

Group	n	CD:UC	CD disease distribution	UC disease distribution	Mean AZA treatment (years)	Mean age (years)	Sex (M:F)	Mean AZA dose (mg/kg)	Mean lowest neutrophil count
Non-neutropenic ($> 2.5 \times 10^9$)	129	79:50	TI = 15 Colitis = 50 Ileocolonic = 14	Pan colitis = 13 Left-sided = 20 Proctitis = 17	4.8	38	49:80	1.9	3.2
Neutropenic ($< 2.5 \times 10^9$)	44	16:28	TI = 3 Colitis = 10 Ileocolonic = 3	Pan colitis = 7 Left-sided = 14 Proctitis = 7	5.0	41	17:27	1.8	1.9

AZA, azathioprine; CD, Crohn's disease; UC, ulcerative colitis.

MWT). For CD patients ($M = 44$, $F = 51$), the neutropenic patients (mean azathioprine dose 1.75 mg/kg, SD 0.43; $M = 6$, $F = 10$) had a mean relapse rate of 0.20/year follow-up (SD = 0.32) compared with 0.23/year follow-up (SD = 0.44) in the non-neutropenic group (mean azathioprine dose 1.95 mg/kg, SD 0.45; $M = 38$, $F = 41$; $P = 0.41$ MWT). Figure 1 shows cumulative remission percentages in UC and CD using Kaplan–Meier survival curves. There is no statistical difference between neutropenic and non-neutropenic groups (chi-squared 0.76 for UC, 0.33 for CD; $P = \text{NS}$). These results are summarized in Table 2.

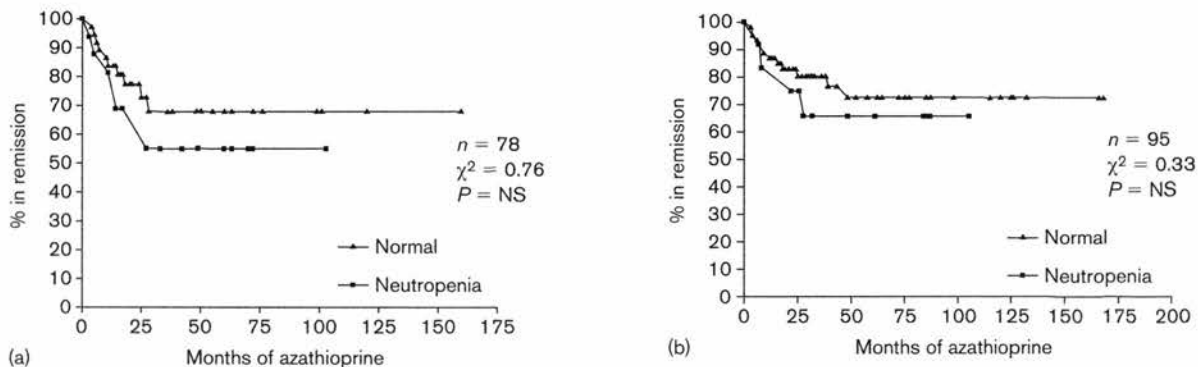
For all IBD patients ($M = 66$, $F = 107$), mean relapse rates in the neutropenic group (mean azathioprine dose 1.80 mg/kg, SD 0.41; $M = 17$, $F = 27$) were 0.28/year follow-up (SD = 0.43) compared with 0.19/year follow-up (SD = 0.37) for the non-neutropenic group (mean azathioprine dose 1.90 mg/kg, SD 0.47; $M = 49$, $F = 80$). This was not statistically significant ($P = 0.37$, Mann–Whitney test). Figure 2 shows cumulative survival curves for IBD patients with no statistical difference between neutropenic and non-neutropenic groups by log-rank analysis (chi-squared 1.49; $P = \text{NS}$).

In addition, univariate and multivariate analyses were performed in smoking and non-smoking groups. These were not statistically significant in any group.

Discussion

From this cohort of 173 patients, remission maintenance is not statistically different in neutropenic versus non-neutropenic patients. If anything, the survival curves suggest that time to first relapse is shorter for patients who achieved neutropenia (neutrophil count $\leq 2.5 \times 10^9$). Further analysis was performed using $\leq 2 \times 10^9$ as the cut-off for the neutropenic groups (data not shown); again, no significant differences in relapse rates were found. In addition, we analysed the lowest neutrophil counts within the first 4 months of therapy (data not shown). This correlated well with the nadir neutrophil count (Pearson correlation coefficient $r = 0.7$; $P < 0.005$), again supporting the theory that neutropenia is not purely a sporadic phenomenon. In view of the fact that neutropenia is not a sporadic phenomenon, in our study we have used not the mean of neutrophil counts, but the lowest neutrophil count. The lowest neutrophil count reflected, in general, the pattern of neutrophil counts closely.

Fig. 1



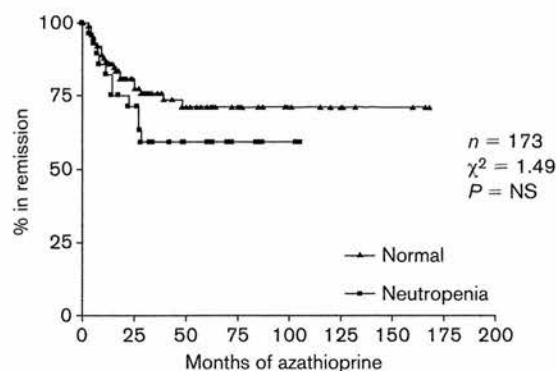
Kaplan–Meier survival curves for (a) ulcerative colitis patients and (b) Crohn's disease patients.

Table 2 Relapse rates for inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's disease (CD)

Group analysis	n	Mean relapse rate/ year follow-up	Range (relapse rate)/year follow-up	SD	MWT (non- neutropenic v. neutropenic)	Mean AZA dose (mg/kg)
UC (non-neutropenic)	50	0.18	0–1.0	0.23	$P = 0.41$	1.85
UC (neutropenic)	28	0.30	0–2.0	0.48		1.90
CD (non-neutropenic)	79	0.23	0–2.6	0.44	$P = 0.48$	1.95
CD (neutropenic)	16	0.20	0–1.0	0.32		1.75
IBD (non-neutropenic)	129	0.19	0–2.6	0.37	$P = 0.37$	1.90
IBD (neutropenic)	44	0.28	0–2.0	0.43		1.80

AZA, azathioprine; MWT, Mann–Whitney Test; SD, standard deviation.

Fig. 2



Kaplan-Meier survival curve for inflammatory bowel disease patients.

Azathioprine immunosuppression is probably mediated through its effects on lymphocytes, although there appears to be preferential suppression of neutrophils when leucopenia occurs [7]. This suggests that azathioprine's anti-inflammatory actions are mediated through its effects on neutrophils. Indeed, it has been shown that neutrophil migration into the lamina propria occurs during clinical relapse in IBD [9]. An anecdotal report has described prolonged remission in excess of 4 years following induced pancytopenia [8]. This report compares well with our own experience, where three of the four patients that developed severe neutropenia have remained in remission for up to 21 months following that episode. More interestingly, the three patients achieving remission all developed sepsis necessitating hospital admission for treatment.

Probably the best documented study looking at the role of leucopenia is by Colonna and Korelitz [5], who studied 98 CD patients retrospectively. They concluded that patients achieving leucopenia were more successful in reducing their CCFA-IOIBD (Crohn's and Colitis Foundation of America/International Organization of Inflammatory Bowel Disease) index, and in maintaining or inducing clinical remission. In addition, these patients were more able to reduce their corticosteroid dose. However, this study had a relatively short period of follow-up (about 18 months), and did not look specifically at neutropenia.

Our study involves a large number of patients over a long period of follow-up of up to 21 years. Despite this being a retrospective analysis, hence subject to type-II errors, this study has an 80% power to detect a 30% difference between the two groups. Thus it could be argued that a smaller difference between the two groups may not have clear implications considering the potential risks of neutropenia. Since neutropenia can

occur at any time during treatment, and thus is relatively unpredictable, a randomized study would prove extremely difficult to perform.

In conclusion, it appears that azathioprine dose titration to achieve neutropenia is not necessary for optimal therapy in IBD patients. Neutropenia $\leq 2.5 \times 10^9$ while on azathioprine does not reduce the relapse rates of IBD patients who were established on azathioprine therapy compared with neutrophil counts $> 2.5 \times 10^9$.

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Effective maintenance of inflammatory bowel disease remission by azathioprine does not require concurrent 5-aminosalicylate therapy

Simon Campbell and Subrata Ghosh

Objectives To assess the effect of 5-aminosalicylate treatment in conjunction with azathioprine on remission maintenance in inflammatory bowel disease patients.

Method This retrospective study was based on a total of 186 inflammatory bowel disease patients (104 with Crohn's disease; 82 with ulcerative colitis), who were stable on azathioprine for a minimum of 6 months. The median duration of follow-up was 4.3 years (range 0.6–15.5 years). Relapse rates per year of follow-up were compared in an azathioprine + 5-aminosalicylate group ($n = 103$) and an azathioprine alone group ($n = 83$); survival curves for cumulative remission rates were compared by log-rank test. Discontinuation of azathioprine in both groups was also recorded, as was the incidence of malignancy.

Results In ulcerative colitis patients ($n = 82$), mean relapse rates for the azathioprine + 5-aminosalicylate group were 0.21/year compared with 0.19/year for the azathioprine alone group ($P =$ not significant). In Crohn's disease patients ($n = 104$), mean relapse rates for the azathioprine + 5-aminosalicylate group were 0.27/year compared with 0.3/year for the azathioprine alone group ($P =$ not significant). The cumulative remission percentage (determined from time to first relapse) was used in Kaplan–Meier survival analysis and showed no difference between the azathioprine + 5-aminosalicylate group and

the azathioprine alone group by log-rank analysis, for ulcerative colitis and Crohn's disease as well as for all inflammatory bowel disease patients. Concurrent use of 5-aminosalicylates was no more frequent in patients who discontinued azathioprine due to adverse events. The only malignancy recorded was Waldenström's macroglobulinaemia after 7 years of azathioprine therapy.

Conclusion Concurrent use of 5-aminosalicylate drugs did not reduce the relapse rates of inflammatory bowel disease patients who were established on azathioprine therapy. The use of 5-aminosalicylate drugs did not lead to any increase in discontinuation of azathioprine due to adverse events. *Eur J Gastroenterol Hepatol* 13:1297–1301 © 2001 Lippincott Williams & Wilkins

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Introduction

Azathioprine (AZA) therapy is an extremely valuable second-line therapy in inflammatory bowel disease (IBD) patients who are refractory to or dependent on steroid therapy. The optimal drug therapy in patients on AZA still remains unclear, with a considerable percentage of patients remaining on a 5-aminosalicylate (ASA) derivative as well as AZA. Some may also require low-dose corticosteroid therapy.

The concomitant use of 5-ASA with AZA has a potential advantage in reducing the long-term colorectal malignancy risk associated with IBD. On the other hand, there is rising concern over the possible interaction of 5-ASA compounds and their breakdown products with thiopurine methyltransferase (TPMT) – the enzyme used in the handling of both AZA and 6-

mercaptopurine. In-vitro studies have suggested that 4-ASA, 5-ASA and sulphasalazine all inhibit TPMT activity to differing degrees, but as yet no studies have looked at the clinical impact and side-effect profiles in patients who take AZA and concurrent 5-ASA drugs.

It is not known whether concomitant 5-ASA therapy is required in patients who remain in remission with AZA. It is possible that 5-ASA therapy may be of additional benefit in maintaining remission in ulcerative colitis patients, but it is unlikely to have such a role in Crohn's disease. In a retrospective study, we compared the relapse rates and AZA discontinuation rates in ulcerative colitis and Crohn's disease patients who remained in remission on AZA alone with those in patients maintained on AZA and a 5-ASA drug.

Patients and methods

From our database of IBD patients maintained on AZA ($n = 220$; ulcerative colitis = 94, Crohn's disease = 126; median age 39 years, range 15–82 years; 105 men, 115 women), we retrospectively examined relapse rates per year of follow-up (i.e. total number of relapses divided by duration of follow-up). All case notes were examined by one investigator (SC). Relapse was defined as a requirement for surgery or documented symptoms consistent with a relapse necessitating rescue medication such as corticosteroid therapy. Thirty-four patients (15%) had stopped AZA due to side effects (dangerous blood dyscrasias, pancreatitis, nausea, abdominal pain, skin rashes or myalgia), 17 patients developed side effects late into AZA therapy before discontinuation, and the remaining 17 patients encountered side effects within the first 6 months of treatment and were never stabilized on AZA – this latter group were excluded from further analysis. This left a total of 186 patients (82 with ulcerative colitis: 44 men, 38 women; median age 43 years, range 15–81 years; 104 with Crohn's disease: 45 men, 59 women; median age 41 years, range 17–82 years) who were stable on therapy for a minimum of 6 months and in remission. The median duration of follow-up was 4.3 years (range 0.6–15.5 years). In addition, we examined information on 34 patients who had to discontinue AZA due to adverse side effects, and assessed whether the patients were on a 5-ASA drug at the time of discontinuation.

Fifty-five patients with ulcerative colitis and 48 patients with Crohn's disease, who were taking AZA for a minimum of 6 months, were also on a concomitant 5-ASA drug (mean AZA dose for ulcerative colitis patients = 1.75 mg/kg, range 0.8–3.5 mg/kg; mean AZA dose for Crohn's disease patients = 1.70 mg/kg, range 0.8–2.7 mg/kg). Twenty-seven patients with ulcerative colitis and 56 with Crohn's disease were taking AZA alone (mean AZA dose for ulcerative colitis patients = 1.85 mg/kg, range 1.0–2.7 mg/kg; mean AZA

dose for Crohn's disease patients = 1.8 mg/kg, range 0.6–3.0 mg/kg). The median 5-ASA doses were 2.4 g/day (range 0.8–3.2 g/day; $n = 71$) of mesalazine (Asacol, GlaxoSmithKline Beecham Pharmaceuticals, Welwyn Garden City, Herts, UK; and Pentasa, Ferring Pharmaceuticals Ltd, Feltham, Middlesex, UK) and 1.5 g/day (range 1–4 g/day; $n = 28$) of sulfasalazine. Two patients were taking 7.5 g/day balsalazide and two patients 1.75 g/day olsalazine. The mean dose of AZA, duration of therapy, frequency of low-dose corticosteroid use, mean neutrophil counts, disease anatomy and frequency of surgery were comparable in the two groups. These results are summarized in Table 1.

Statistical analysis was by Student's *t*-test for comparing continuous variables between the two groups (AZA alone and AZA + 5-ASA) in ulcerative colitis and Crohn's disease patients separately. Categorical data were compared using the chi-squared test with Yates' correction. Kaplan–Meier survival curves were constructed using the time to first relapse and compared between the two groups using the log-rank test.

Results

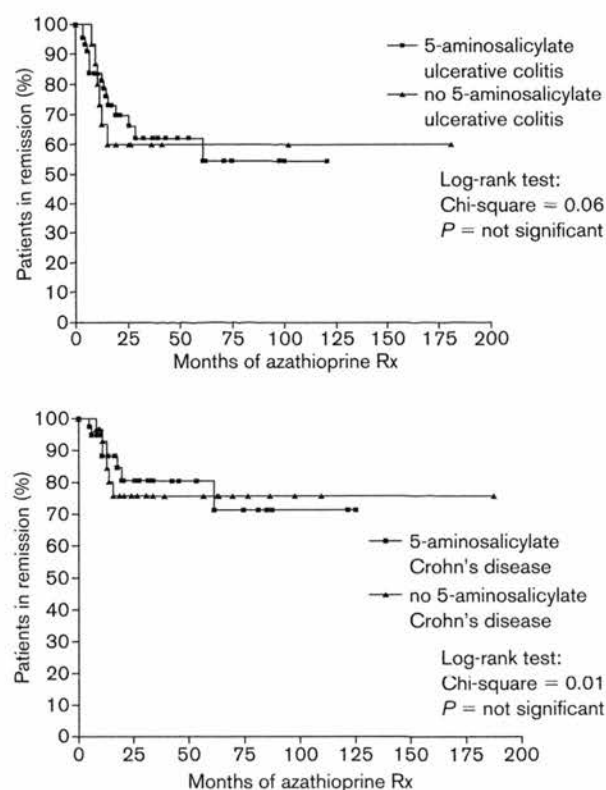
A total of 186 IBD patients were stable on AZA for a minimum of 6 months. The mean duration of therapy was 4.3 years (standard deviation = 3.0). Of these patients, 103 (55%) were taking concurrent 5-ASA drugs.

Analysis was performed for ulcerative colitis and Crohn's disease patients separately. In the ulcerative colitis patients ($n = 82$), the AZA + 5-ASA group ($n = 55$) had a mean relapse rate of 0.21/year of follow-up (standard deviation = 0.31) compared with the AZA alone group ($n = 27$), who had a mean relapse rate of 0.19/year of follow-up (standard deviation = 0.28; $P = 0.69$; Student's *t*-test). In the Crohn's disease patients ($n = 104$), the relapse rate of the AZA + 5-ASA group ($n = 48$) was 0.27/year of follow-up (standard deviation = 1.0) while the relapse rate of the AZA alone

Table 1 Patient characteristics (disease distribution, steroid use, azathioprine therapy duration and neutrophil counts)

Group	<i>n</i>	Disease distribution	Mean duration of azathioprine (AZA) therapy (years)	Mean age (years)	Male to female ratio	Mean AZA dose (mg/kg) (range)	Number of patients taking low-dose corticosteroid	Number of patients having surgery	Mean neutrophil count
AZA+5-aminosalicylate (ASA) (ulcerative colitis)	55	Pan colitis = 19 Left sided = 18 Proctitis = 18	4.7	45	30:25	1.75 (0.8–3.5)	11	2	3.8
AZA+5-ASA (Crohn's disease)	48	Terminal ileum = 8 Colitis = 31 Ileocolonic = 9	4.0	40	15:33	1.70 (0.8–2.7)	10	4	4.3
AZA alone (ulcerative colitis)	27	Pan colitis = 11 Left sided = 8 Proctitis = 8	4.3	43	14:13	1.85 (1.0–2.7)	4	2	4.7
AZA alone (Crohn's disease)	56	Terminal ileum = 15 Colitis = 26 Ileocolonic = 15	4.6	38	30:26	1.80 (0.6–3.0)	14	5	4.9

Fig. 1



(a) Remission percentage based upon time to first relapse for ulcerative colitis patients. (b) Remission percentage based upon time to first relapse for Crohn's disease patients.

group ($n = 56$) was 0.30/year of follow-up (standard deviation = 0.8; $P = 0.97$; Student's t -test). Figure 1a shows the remission percentage based upon the time to first relapse for ulcerative colitis patients; Figure 1b shows the same analysis in Crohn's disease patients. There was no statistical difference between patients on AZA alone and those on AZA + 5-ASA in either disease by log-rank test ($\chi^2 = 0.06$ for ulcerative colitis patients and 0.01 for Crohn's disease patients; $P =$ not significant). These results are summarized in Table 2.

In those patients who had to discontinue AZA therapy due to side effects ($n = 34$), there was no increase in frequency of 5-ASA therapy ($n = 15$; number discontinued in AZA alone group *vs.* number discontinued in AZA + 5-ASA group: $\chi^2 = 0.4$; $P =$ not significant). In addition, the 17 patients who discontinued AZA before stabilization (i.e. before 6 months) showed no significant differences in side effect types or mean dose of AZA from those patients who discontinued AZA after 6 months ($n = 17$). This is summarized in Table 3.

Finally, there were no cases of malignancy in this group, although one patient with Crohn's disease developed Waldenström's macroglobulinaemia after 7 years of AZA and mesalazine therapy, and two patients with ulcerative colitis developed low-grade dysplasia, one after 5.8 years of treatment with AZA and sulfasalazine and the other after 3 years of treatment with AZA and mesalazine.

Discussion

Relapse rates per year of follow-up were not statistically different between the AZA + 5-ASA group and the AZA alone group (in either ulcerative colitis or Crohn's disease patients) and our cumulative remission curves compare well with other studies which have looked at remission maintenance with 5-ASA and AZA [1-6]. It would therefore appear that 5-ASA compounds afford no additional clinical benefit on relapse rates in patients suffering from either ulcerative colitis or Crohn's disease. This study used data from a large database of IBD patients who were well matched for disease type and distribution, use of low-dose corticosteroids and frequency of surgery; there were comparable numbers of patients taking, and not taking, 5-ASA compounds (Table 1).

However, this is a retrospective analysis of two groups of patients and may be selectively biased. It could be suggested that patients taking AZA alone had a more aggressive disease, which was treated at an early stage with AZA rather than using 5-ASAs. However, patients who were taking 5-ASA in addition to AZA did not have a more aggressive disease course in terms of

Table 2 Mean relapse rates for patients taking azathioprine (AZA) + 5-aminosalicylate (ASA), and for patients taking AZA alone

Group analysis	<i>n</i>	Mean relapse rate/year follow-up	Range (relapse rate)	Standard deviation	Student's <i>t</i> -test (AZA+5-ASA vs. AZA alone)	Mean AZA dose (mg/kg)
Ulcerative colitis (AZA + 5-ASA)	55	0.21	0-1.3	0.31	$P = 0.69$	1.75
Ulcerative colitis (AZA alone)	27	0.19	0-0.9	0.28		1.85
Crohn's disease (AZA + 5-ASA)	48	0.27	0-7.5	1.0	$P = 0.97$	1.7
Crohn's disease (AZA alone)	56	0.30	0-5	0.8		1.8

Table 3 Comparison of patients who discontinued azathioprine (AZA) therapy before and after 6 months into treatment

	n	Male to female ratio	Crohn's disease to ulcerative colitis ratio	Taking 5-amino-salicylate?	Mean AZA dose (mg/kg)	Mean duration of AZA therapy (years)	Side effects				
							Rash	Pancreatitis	Malignancy	Abdominal pain/nausea	Hepatitis
Discontinued after 6 months	17	7:10	10:7	8	1.6	4.4	2	2	1	4	3
Discontinued before 6 months	17	9:8	12:5	7	1.4	0.2	4	4	0	3	3

previous relapses, anatomical extent of disease or their requirement for surgery than those taking AZA alone. It was not possible to elucidate the impact of 5-ASA compounds on long-term malignancy risks in these patients because of the retrospective nature of the study. Although there are recent studies advocating the protective role of 5-ASA drugs in the development of colorectal malignancy [7–11], it still remains uncertain whether this is a direct effect of the 5-ASA drugs or merely the result of prolonged disease remission. Interestingly, there have been reports of the potential role of folate supplementation in patients taking sulfasalazine (a competitive inhibitor of folate absorption) to reduce the incidence of dysplasia and cancer in chronic ulcerative colitis [12]. The role of 5-ASAs, especially sulfasalazine, in the prevention of colorectal malignancy is controversial and currently lacks firm evidence. It is worthy of note that one case of dysplasia reported here was in a patient taking sulfasalazine; there was no obvious excess occurrence of malignancy in our group of patients taking this drug.

There has been growing interest over the last few years in the possible interaction between 5-ASA compounds and the enzyme TPMT. It has been reported that TPMT is potently inhibited by derivatives of benzoic acid [13]. Indeed, there is convincing evidence that it is the 3-, 4-, and 5-ASA components, rather than other carrier molecules involved in the drug interaction, that inhibit TPMT [14]. In-vitro analysis has highlighted significant interactions with TPMT and balsalazide [15], olsalazine [15] and sulfasalazine [16], and it is likely that mesalazine has a similar effect, though there is a lack of data on this drug. In our study, the majority of patients were taking mesalazine, with smaller numbers taking sulfasalazine, balsalazide and olsalazine, and it would appear that there is no evidence of any clinical risk from this potential drug interaction. Clearly, caution should be exercised whenever 5-ASA drugs are being used in conjunction with AZA or 6-mercaptopurine, especially if more potent inhibitors such as olsalazine and balsalazide are being used. The importance of TPMT inhibition by these drugs will be especially important in the minority of patients (up to 10%) who have genetically low levels of TPMT.

In summary, it is now important to follow up this study with a prospective double-blinded and placebo-controlled study to further validate these results.

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